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The sex ratio of mice from alcoholized fathers.

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Bluhm[1] and Danforth,[2] using somewhat different experimental methods, have reported a rise in the sex ratio of mice from alcohol treated fathers. However, these ratios are based on small numbers (182 ♂ : 149 ♀ Bluhm; 210 ♂ : 164 ♀ Danforth) and, although the probable errors of the deviations from the control ratios (+10.60 per cent ±2.03 and +5.36 per cent ±1.99) may be taken to mean that random sampling alone is not responsible for the results, various other possible influences besides alcohol, such as season, mother's age and parity, have not been eliminated by the methods of these investigators. Moreover, Gyllenswärd's[3] more completely controlled experiments with alcoholized male mice show as great a change in the opposite direction (—10.4 per cent ±3.4), and MacDowell and Lord[4] have called attention to the fact that when all question of the modification of the sex

[1] Bluhm, A., *Arch. Rass. Gesel. Biol.*, 1924, xxvi, 1.

[2] Danforth, C. H., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 305.

[3] Gyllenswärd, C., *Bidrag Till Fragan om Alkoholverkningars Ärftlighet.*, Stockholm, 1923.

[4] MacDowell, E. C., and Lord, E. M., *Anat. Rec.*, 1925, xxxi, 143.

ratio by prenatal mortality was removed by using only complete litters (so judged by the number of the corresponding corpora lutea) the primary sex ratio given by litters from heavily alcoholized fathers and normal mothers (50.3 per cent males, based on 308 mice) showed no significant deviation from the primary sex ratio from normal parents (49.9 per cent males, based on 523 mice).

The present report gives the sexes of 2133 mice from normal mothers by fathers given completely anesthetizing doses of alcohol fumes five days a week, beginning at the age of four weeks and continued over a year, to the end of the experiment; and 2322 mice from the same normal mothers by normal fathers, brothers of those treated. The treatment was given by inhalation, in one pint milk bottles; for each treatment 3 cc. 95 per cent alcohol was poured on a piece of absorbent paper which was placed in the bottle with the mouse, a regular milk bottle cap inserted and the bottle inverted. The time necessary to produce complete anesthesia varied; 45 minutes for the mice of four weeks, for adults, depending on the condition of the atmosphere and the mouse, one to two hours. The control males were bottled in the same way at the same times, but with no alcohol.

Comparability of the treated and control fathers was obtained by using four males from the same litter as the unit experiment; two of these, chosen by a system ensuring random selection, were treated, the other two used as controls. Uniformity of conditions of gestation and other influences of the mothers was ensured by mating each female (16-20 in each experiment) alternately with a treated and control male. All females were from the same inbred line and within two weeks of the same age, three to four weeks older than the males in the experiment. The females in each litter were equally divided between treated and control males for their first matings; subsequent matings by the other males in rotation, controls always alternated with treated males. The young were killed and sexed on the day of birth and the mother mated at once with the next male. Four unit experiments were undertaken in which males from the Bagg albino line were used, and four experiments in which males from the Dilute-brown line were used.

Table I gives a summary of the results with the probable errors for the deviations from a 1:1 ratio. The totals from the four experiments with the Bagg albino males show a slight excess of

males from the controls and a slight excess of females from the treated fathers. The four experiments with the Dilute-brown males give a slight excess of females from the controls and a slight excess of males from the treated fathers. In no case does the deviation from equality even approach statistical significance. Unless some unrecognized influence has not been controlled in these experiments the results seem conclusive that the treatment of the males with heavy doses of alcohol fumes has not modified the sex ratio.

TABLE I.

	From treated fathers			From control fathers		
Line of father	No. of mice	Per cent males	Deviation from 50 %	No. of mice	Per cent males	Deviation from 50 %
B. alb.	1261	48.69	—1.31±0.95	1283	51.60	+1.60±0.94
D-br.	872	50.57	+0.57±1.14	1039	49.76	—0.24±1.05
Total	2133	49.46	—0.54±0.72	2322	50.77	+0.77±0.70

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The vitamin content of oysters.

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A twofold interest is connected with a study of the vitamin content of oysters. They constitute an important and an extensively used item of food. Furthermore, the material upon which they feed consists largely of diatoms and minute organisms, marine forms of life to which have been traced the origin of the fat-soluble vitamins found so abundantly in certain fish liver oils, such as that of the cod.[1]

So far as we are aware, no work has been hitherto reported on the vitamin content of oysters with the exception of that pub-

[1] Hjort, J., *Proc. Roy. Soc.*, Series B, 1922, xciii, 440; Drummond, J. C., and Zilva, S. S., *Biochem. J.*, 1922, xvi, 518.

lished by Randoin[2] on the antiscorbutic factor. They found that oysters contain this vitamin in abundance.

By means of feeding tests with rats, we have found that oysters are rich also in vitamins A and B. In order to obtain uniform samples, fresh oysters were ground in a frozen condition. Quantities of the frozen product equivalent to 0.5 gm., calculated on a dry basis, caused prompt resumption of growth when fed daily to rats that had declined in weight as a result of the lack of vitamin B in their basal ration. Experiments in progress indicate that smaller quantities are sufficient to meet the requirements of rats for this vitamin.

As little as 0.25 gm. of a product obtained by dehydrating fresh oysters at a temperature not exceeding 40° under reduced pressure, enabled rats to make a fair recovery from the results of vitamin A deficiency.

It was found that during the process of dehydrating the oysters a change took place which caused a partial destruction of vitamin B. Whether this process also impaired the vitamin A value of the oysters is being investigated. Work is also in progress to estimate the vitamin content of clams, shrimp and other articles of sea food.

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The photo-electric cell as a colorimeter.

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The photo-electric cell determines the intensity of illumination to which it responds, by means of varying amperage, which it allows to pass through under the stimulus of an E.M.F. Any change in the illumination can be determined by changes in this amperage. The variables in an apparatus set up for this determination are: (a) the voltage offered to the cell from "B" batteries or other source of current, such as a "B eliminator," or other

[2] Randoin, L., *Compt. Rend. Acad. Sci.*, (Paris), 1923, clxxvii, 498.

suitable control for voltage, (b) the distance of the light source from the cell, (c) the wattage of the light source, (d) the concentration of the solution to be analyzed, as, for example, standards of 100 or 200 milligrams of sugar, in which the blue color has been developed by the ordinary Folin-Wu procedure, (e) the height of the column of liquid through which the light passes, (f) the area of the cross section of the solution, etc.

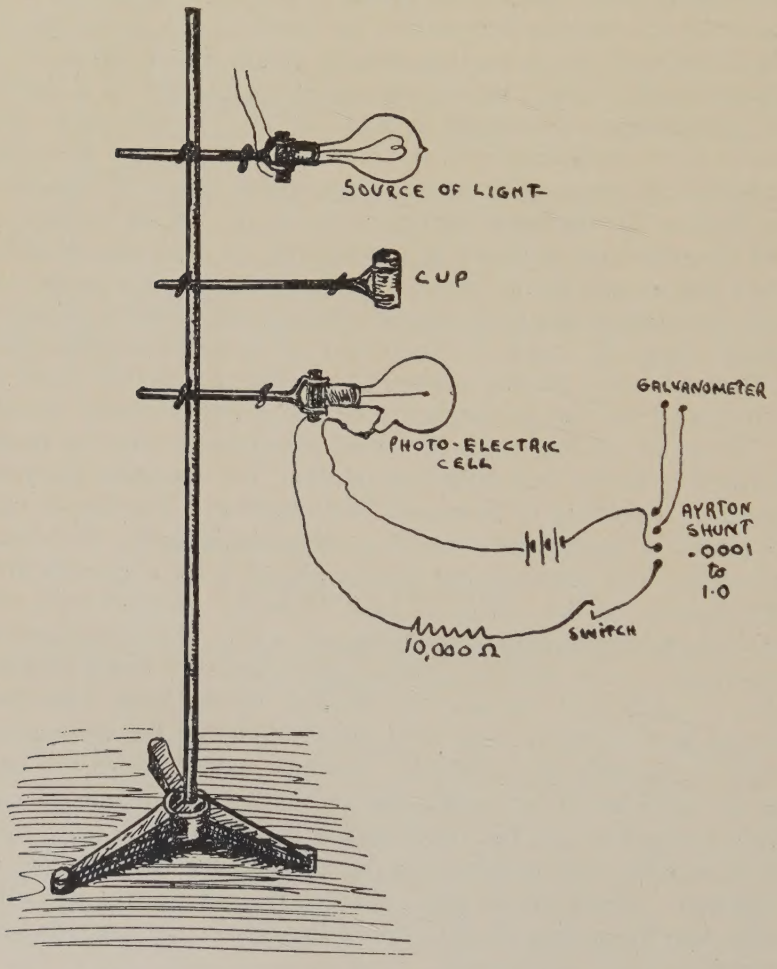
All of these variables when plotted, do not give straight lines throughout wide ranges, but all do over certain distances. By adopting the straight line part of the curves in the actual use of the instrument it has been possible to obtain direct and indirect proportional results. Briefly, the apparatus consists of a source of illumination, an ordinary bulb of 25, 40, 75, 100, up to 250 watt power, depending upon the conditions adopted for the other variables; a source of constant voltage either from "B" batteries or from a "B eliminator" plugged into an A C house current; a cell to receive the solutions to be analyzed, for example, an ordinary cup as used in the Duboscqe colorimeter; the photo-electric cell; and an instrument to measure the current, either a galvanometer (type R, Leeds & Northrup) or a micro-ampere-meter (Weston). For further control of readings an Ayrton shunt is introduced into the galvanometer or micro-ampere-meter circuit.

There are several ways in which comparisons may be made between a known and unknown solution, for example, dextrose prepared according to Folin and Wu's method. The known can be placed in the apparatus and a reading obtained, say 40 micro-amperes. The unknown can then be placed in the apparatus and the distance of the source of light from the photo-electric cell varied until the reading of the unknown is also 40 micro amperes, whereupon, when suitable conditions are adopted, a direct proportion is established between the distance of the light when the known and unknown were read; this determines the amount of sugar in the unknown solution. Another way in which this can be accomplished is by varying the heights of the column of unknown solution until the same reading is obtained. A third way is to vary the voltage offered to the cell until the same reading is obtained. There are several other ways, but of five methods tried, that of varying the distance of the light source proved most convenient and satisfactory.

The method has been tried with colored substances from the red to the blue end of the spectrum; the comparative results

between the unknown and known have been accurate in all ranges, so that the cell is useful throughout the entire range of the spectrum. As an example of its accuracy, it has been possible to determine the difference constantly, and by at least five separate individuals operating the apparatus independently, between 100 and 101 milligrams of sugar per 100 cc. of solution. Since only 5 cc. of solution are used for the actual determinations the accu-

FIG. 1.



One possible set-up and hook-up of the apparatus. Care must be taken by means of shields, etc., to exclude all extraneous light.

racy of the instrument seems quite as good as a chemical balance, that is, between 0.005 and 0.00505 milligrams.

To be emphasized is the fact that not only are the results consistent when one operator is at work, but at least five in our laboratory have checked analyses to the fifth decimal place. From other experiments at hand, it seems quite probable with suitable apparatus that this accuracy can be increased to an even greater extent. The personal factor of color comparisons is absolutely eliminated. A child can operate the instrument.

Further experiments have been made toward the reading of spectroscopic, spectro-photometric, polaroscopic and microscopic differences in color and illumination intensity. Furthermore, it has been used as an nephelometer with excellent results. Other fields of usefulness have suggested themselves and are steadily being investigated.

Full details, including curves, will be published shortly along with a detailed description of a convenient assembly of parts. Many pitfalls must be avoided and these will then be fully discussed.

SUMMARY.

Methods are described of using the photo-electric cell, in our case an argon filled bulb, whereby colorimetric and nephelometric determinations can be made with exceeding accuracy, thus threatening to displace the use of the ordinary Duboscqe and other similar colorimeters. The diagram indicates one possible set-up of the instrument and one possible hook-up whereby the measurements can be made. Others can be evolved, as we have determined; in fact, it is possible to measure color by sound after suitably treating the current obtained and passing it through amplifying devices into a loud speaker or a pair of ordinary radio head phones. This should give much finer readings than even by the use of the type R galvanometer which determines 0.000,00025 amperes.

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Studies on the filterability of mouse sarcoma.

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Rous[1] first pointed out that he was able to transmit the sarcoma of the Plymouth Rock hen to other fowls of the same species by means of a tumor extract which had been rendered cell free by passage through a Berkfeldt filter. Numerous attempts to obtain a similar filterable extract from mammalian tumors were uniformly negative until Gye[2] and Barnard[3] published their reports concerning the filterability of the mouse as well as the Rous chicken sarcoma. In their exhaustive studies of these filtrates they suggest the possibility, and offer experimental data to prove that the infective nature of the Rous tumor depended upon the adjustment of two agents: one, a filterable ultra-microscopic virus, common to all tumors, the other, an accessory or unstable chemical substance derived from the tissue itself, which they called the "specific factor." It was deemed advisable to repeat their experimental work, especially upon the mammalian tumors.

For the first point of attack, mouse sarcoma No. 37 was selected, which was the original tumor Gye and Barnard worked with at the laboratory of the National Research Institute. The tumor is a spindle cell sarcoma, grows very rapidly in practically 100 per cent, is very cellular, with very slight central necrosis, and attains full growth within 10 to 12 days.

Experiment No. 1: the tumor-bearing mouse was killed with gas, the carcass dipped for a few moments in a 4 per cent lysol solution, and the tumor was then excised by means of a cautery to insure perfect asepsis. One to two grams of the tumor tissue were placed in a tube containing 5 cc. of Hartley's broth plus 1 cc. of fresh rabbit serum. The tubes were put in a MacIntosh-Fildes jar for anaerobic incubation, the air was evacuated by a motor pump, and hydrogen gas was then allowed to enter the jar, in order to combine with any remaining oxygen under the

[1] Rous, P., *J. Exp. Med.*, 1911, xiii, 397.

[2] Gye, W. E., *Lancet*, 1925, July 18, 109.

[3] Barnard, J. E., *Lancet*, 1925, July 18, 117.

influence of electric sparking. To control complete anaerobiosis, a tube of methylene blue was also placed in the jar to indicate the absence of oxygen. The jar was placed in an incubator at 36.5 degrees C. and left for 23 to 24 hours. In almost every instance the indicator remained colorless, thus proving complete anaerobiosis. The culture tubes were then removed from the jar, and the sterility tested by stab and slant cultures on agar. The tumor cultures were now centrifuged for 10 to 12 minutes at 2700 revolutions per minute and only the supernatant fluid pipetted off, and 1 cc. injected into normal mice.

Several of these experimentally produced tumors have been inoculated into normal mice, and up to the present have gone through nine generations.

To rule out the possibility that an occasional living cell in the supernatant fluid may have given rise to tumor production, the fluid was filtered through a fine Berkfeldt filter. In this manner it was thought to meet the requirements of a cell free fluid, and to test the filterability of Mouse Sarcoma No. 37. With this end in view the following experiment was carried out:

TABLE I.
Injections of Mouse Sarcoma No. 37 Broth Serum Culture.

Cage No.	Date	Animals injected	No. tumors
12	10/28/25	4	3
13	10/29	4	3
15	10/30	4	4
17	11/3	4	2
19	11/6	4	3
21	11/11	6	0
23	11/12	1	0
26	11/14	1	0
41	12/5	10	2
45	12/9	11	8
46	12/10	11	2
48	12/14	7	0
85	1/19/26	1	0
102	2/2	3	1
104	2/2	3	0
106	2/5	2	0
108	2/6	4	1
110	2/11	1	0
112	2/13	5	0
114	2/16	4	1
116	2/18	4	2
117	2/20	3	2
120	2/23	4	0
Totals		105	34

Experiment No. 2: the tumor was excised aseptically and placed in 5 cc. tubes of Hartley's Broth plus rabbit serum, and incubated under anaerobic conditions for 23 to 24 hours. The tubes were then removed from the jars, and the fluid from these cultures filtered through Berkfeldt Filter "N", which had been tested against *B. prodigiosus.* The filtrate thus obtained was injected subcutaneously in doses varying from 1 cc. to 2 cc. into 164 mice, with 6 successful tumor productions.

TABLE II.
Injections of Filtrate of Mouse Sarcoma No. 37.

Animals injected	Tumor takes	Percentage tumor takes
164	6	3.66

To find out whether the anaerobically incubated tumor tissue was still alive after 24 hours incubation and capable of proliferation, the incubated tumor was removed and washed free of the serum broth by centrifuging it for 30 minutes in 3 changes of Ringer's or Tyrode's solution. The usual size fragments of this incubated tumor tissue were then inoculated into 43 normal mice, with but one successful "take". In the instances where the washing of the incubated tissue was omitted, there were two takes out of 38 inoculations. In other words it is safe to assume from these experiments that the tumor tissue itself is not sufficiently viable to account for the usual 30 per cent of takes when the centrifuged supernatant fluid of the tumor cultures is injected.

TABLE III.
Inoculation of Anaerobically Incubated Tumor Tissue.

Washed tumor tissue		Unwashed tumor tissue	
No. injected	Takes	No. injected	Takes
43	1	38	2

Experiment No. 3: Other experiments were conducted upon Mouse Carcinoma No. 63, supplied by the Imperial Cancer Research Fund of London. This carcinoma is somewhat hemorrhagic, does not grow as rapidly as the Mouse Sarcoma No. 37, requiring 14 to 21 days for full growth. The same technique of anaerobic cultivation was used with the Mouse Carcinoma

No. 63, as with the Mouse Sarcoma No. 37. After 23 to 24 hours incubation, the tubes were centrifuged for 10 to 12 minutes, the supernatant fluid pipetted off, and 1 to 1.5 cc. of the fluid was injected subcutaneously into 81 mice with 2 "takes".

TABLE IV.

Injection of Broth Serum Cultures of Mouse Carcinoma No. 63.

Mice injected	Tumor takes	Percentage tumor takes
81	2	2.5

Experiment No. 5: We applied the same technique as in Experiment No. 2, namely, we filtered the fluid of the incubated mouse Carcinoma No. 63. Out of 27 subcutaneous injections of the carcinoma filtrate there has not been up to the present a single successful tumor "take". This work, however, is still in progress.

Another point of interest is the time of the first appearance of the tumor nodule. The tumors produced by the injection of the supernatant fluid appeared from the 12th to the 24th day, averaging 17 days. The tumors produced by the injection of the cell-free filtrate of Mouse Sarcoma No. 37 were first observed on the 20th, 18th, 26th, 34th, 20th, and 22nd day respectively, averaging the 23rd day. All experimentally produced tumors upon excision were sectioned and examined microscopically, revealing the typical picture of the original Mouse Sarcoma No. 37.

For the present we do not wish to draw conclusions from our results, as the work had to be carried on under rather adverse conditions. For instance on account of the scarcity of supply of mice at one time during the winter, the mice had to be obtained from different sources, and irregular strains used. This seriously affected the behavior of our stock inoculations. It is only fair to assume that this had some effect upon the experimental work. At another time an incidental epidemic carried off a great many of the normal experimental animals, and therefore we are reporting only those experiments where the mice survived the period of 2 weeks after the experiment was initiated.

CONCLUSIONS.

1. We have been successful in obtaining 34 mouse sarcoma No. 37 out of 105 injections, when supernatant fluid of cultures of Mouse Sarcoma No. 37, incubated anaerobically for 23 to 24 hours, was used.

2. The Berkfeldt filtrate of Mouse Sarcoma No. 37 also incubated anaerobically yielded 6 tumors out of 164 injected animals.

3. Tumor tissue incubated anaerobically does not seem to be sufficiently viable to produce tumors when inoculated subcutaneously.

4. Mouse Carcinoma No. 63 yielded 2 tumors out of 81 injections of the supernatant fluid from incubated mouse carcinomata.

5. The filtered fluid from Mouse Carcinoma No. 63 so far has not produced a single successful tumor formation.

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Functioning autoplastic suprarenal transplants.

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No proof has as yet been presented that free transplants of the suprarenal gland function. Our experiments, with the view of obtaining evidence of function, were carried out on both the rat and the guinea pig. We realize the difficulties of interpreting results in favor of function in the rat, particularly if the clinical condition of the animal is taken as an index, because of the frequent occurrence of accessory suprarenal tissue. For crucial evidence of functioning free transplants we used the guinea pig because continued survival of this species after complete bilateral suprarenal ablation has never been reported. The best results are Rogoff's,[1] who, in a series of 17 animals with an interval of 2 to 3 weeks between the removal of the right and left glands, reports that 1 pig lived 28 days, while 14 of the 17 died during the first 8 days.

PROCEDURE.

In the rat both glands were removed in one sitting and placed in sterile physiological saline at about 39° C. Each gland was cut in half and the four parts were immediately transplanted into

[1] Rogoff, J. M., quoted by Stewart, G. N., *Physiol. Rev.*, 1924, iv, 167.

pockets between the fascia and abdominal muscle, or in the abdominal muscle. In 3 or 4 weeks the transplants regenerate as highly vascularized masses of cortical tissue, with the three cortical layers. Medullary tissue does not regenerate. Transplants in rats sometimes attain the size of suprarenal glands of adult animals.

In the guinea pig difficulties are encountered in transplantation because the muscle of this animal is highly sensitive to epinephrin, as described by Elliott and Tuckett.[2] Small amounts of this drug when introduced with the transplant may cause marked swelling, and this may be followed by sero-sanguinous oedema, necrosis, sloughing, and infection of the abdominal wall, so that the transplants are destroyed. For the successful transplantation of the suprarenal in guinea pigs, the medulla must be separated from the cortex. Our procedure, therefore, is to remove the right suprarenal gland completely, separate cortex from medulla, and transplant 8 to 16 fragments of cortex into the abdominal wall. After an interval of from 3 to 7 weeks the left suprarenal is removed.

EVIDENCES OF FUNCTION.

Rat. The evidence of function of free suprarenal transplants in this animal is suggestive for the following reasons, but not conclusive:

1. Of 67 young rats which were suprarenalectomized and autoplastically transplanted, and observed from 2 to 10 months after operation, only 4 died; in our experience about 40 per cent of these animals would have died if they had been only suprarenalectomized.

2. In every instance where the rat, after transplantation, died spontaneously from suprarenal insufficiency, the transplants had degenerated.

3. Where the transplants were large and well vascularized the rats approached the normal litter and sex controls in weight and activity.

Guinea pig. The right suprarenal was transplanted and the left gland removed in 19 guinea pigs. Five animals died within 4 days after removal of the left gland. There was 1 operative death, and 4 deaths from suprarenal insufficiency, 1 on the 2nd, 2 on the 3rd, and 1 on the 4th day. The autopsies of the last 4

[2] Elliott, T. R., and Tuckett, I., *J. Physiol.*, 1906, xxxiv, 362.

animals showed complete removal of both main glands, no macroscopic accessories, and no positive transplants. In 2 of these 4, the marking sutures were missing, due either to sloughing of the abdominal wall or to absorption.

Of the remaining 14 guinea pigs, 8 are still surviving in good condition, 2 for 40 days and the rest for 60 days after the removal of the second gland. These animals are active and eating, although 1 is not gaining in weight. Two were sacrificed in good condition 14 days after removal of the left gland; the autopsies showed complete bilateral ablation of the suprarenals, and numerous pin-head sized positive transplant areas, which on section showed nests of well vascularized cortical cells in glomerular formation. Four guinea pigs died from suprarenal insufficiency following removal of the left gland, the deaths occurring on the 15th, 41st, 48th, and 52nd day. Two of these animals were pregnant. The transplants in these animals had been absorbed.

Guinea pigs do not live on an average for more than 3 or 4 days after removal of both suprarenal glands. We have 8 out of 12 transplanted guinea pigs surviving from 40 to 60 days after complete removal of both suprarenal glands. These results, therefore, bring strong evidence in favor of the fact that the transplants are maintaining life.

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Purification of cultures of bacteria by means of reverse selective bacteriostatic properties of aniline dyes.

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In a recent study of the sporulation of *Bacillus anthracis,* this organism was recovered from the spleen and heart's blood of a mouse, dead of the experimental disease, in association with a small gram negative bacillus. The presence of the contaminating organism could not be detected on the agar transplants because of the overgrowth of *B. anthracis,* but it could readily be seen in smears. The observation of this gram positive spore bearing

bacillus with a gram negative bacillus presented an excellent opportunity to test the validity of the idea that in such cases purification of the cultures could be effected on the principle of reverse selective bacteriostasis. If the observations previously reported on this subject were correct, it would be possible in the case of this particular mixture of *B. anthracis* and a gram negative bacillus, to isolate either of the two organisms, in pure culture, by simply treating the mixture with one of two dyes, whose selective bacteriostatic properties, as between organisms of these two types, were known to be opposite in character.

As has been previously reported,[1] gentian violet and acid fuchsin possess these opposite selective properties. The culture of *B. anthracis* contaminated with the gram negative organism (which we may call *Bacillus X*), was therefore stroked in a heavy suspension across a divided gentian violet plate. The upper half contained dye in a 1/100,000 dilution. As had been expected, *Bacillus X* grew out in pure culture on the gentian violet half of the plate. Smears made from this culture showed practically nothing but *Bacillus X*, although an occasional individual *B. anthracis* could be seen, probably dead. An absolutely pure culture could be readily obtained by again stroking on another gentian violet plate an aqueous suspension of the growth obtained from the gentian violet half of the divided plate. It was thus very easy to rid the culture containing *B. anthracis* and *Bacillus X* of *B. anthracis* by growing it on gentian violet. The attempt was then made to rid the mixture of *Bacillus X* and obtain *B. anthracis* in pure culture. For this purpose to ½ cc. of an aqueous suspension of the contaminated culture, ½ cc. of one per cent acid fuchsin (Grübler) was added. These tubes, together with control tubes of a similar suspension containing no stain, were placed in a water bath and kept at 45 degrees for one hour. Streaks were then made on plain agar. All the controls grew well, clearly indicating that the slight amount of heat used had not injured either organism. In the tube containing acid fuchsin no *Bacillus X* appeared, a pure culture of *B. anthracis* being demonstrated by smears. It has thus been possible by the use of acid fuchsin to effect a selective purification of a contaminating culture opposite in character to that produced when the mixture was exposed to the bacteriostatic effect of gentian violet.

[1] Churchman, J. W., *J. Exp. Med.*, 1923, xxxvii, 1-10.

After these two organisms had been thus obtained in pure culture by the method of selective bacteriostasis, the selective susceptibility of each organism to the two dyes used, was then tested out. One half cc. of aqueous suspension of pure cultures of each of the two organisms were placed in test tubes. To one series of these suspensions a small platinum loop full of 1 per cent gentian violet was added, and to another series ½ cc. of 1 per cent aqueous acid fuchsin (Grübler). The gentian violet tubes were kept at room temperature, the acid fuchsin tubes at 45 degrees. At the end of an hour streaks from these tubes were made on plain agar, control inoculation of organisms which had not been exposed to dyes being always made at the same time. These experiments clearly showed that gentian violet in the strength used was entirely without effect on *Bacillus X*, though it completely inhibited the growth of *B. anthracis*, while acid fuchsin was entirely without effect on *B. anthracis*, although showing marked and often complete inhibition of *Bacillus X*.

Although I have made very clear in previous publications[2] that the bacteriostatic properties of the dyes are much more important than the bacteriocidal properties, other observers[3] who have repeated my experiments have not always borne this distinction clearly in mind. It should, therefore, be stated again that in the experiments as here described, emphasis is placed chiefly on the static properties of the dye and nothing is said as to their power actually to kill organisms. When bacteria are exposed to dyes and then streaked on plain agar, it is of course, perfectly clear that any result produced may be in part due to the inhibitive power of the dye which is carried over to the agar when the transplant is made. This fact, however, in no way invalidates the principle of reverse selective bacteriostasis which observations of the kind here reported, confirm. This principle is to the effect that in experiments done in such a way as to test the combined bacteriostatic and bacteriocidal powers of the dyes, but not so as to distinguish clearly between these powers, some of the dyes in strengths which inhibit gram negative organisms are without effect on gram positive spore bearing aerobes, while other dyes in strengths which inhibit certain gram positive spore bearing aerobes are without effect on certain gram negative organisms.

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[2] Churchman, J. W., *J. Exp. Med.*, 1912, xvi, 221.

[3] Burke, V., and Skinner, C. E., *J. Exp. Med.*, 1925, xli, 471.

The acid fuchsin used in these experiments came from a Grübler specimen which had been present for a long time in the laboratory. This particular sample of dye has been compared as regards its bacteriostatic property for *Bacillus X* with one other sample of Grübler dye and also with two samples of dye obtained from the Wills Corporation of Rochester, N. Y. 542, 45 and 524, 902. It has also been compared with neutral acriflavine. The experiments showed these substances also to possess a reverse selective power, though the sample first used (that is to say the Grübler dye) was more potent than any of the others. Certain observers[4] have reported that they have been unable to produce any static effect on bacteria by the use of acid fuchsin. The variation in the results which I obtained with different samples of this dye indicates that many substances of varying composition have probably been sold under the name of acid fuchsin and the variability in the results may be due to this fact.

The organism called *Bacillus X* was not absolutely identified. As the cultures from the animals dead of experimental anthrax, in which the contaminated *Bacillus X* appeared, were made a few hours after the death of the animals, it seemed quite likely that *Bacillus X* had invaded the blood and tissues from the intestine. The organism had the following characteristics:

Morphology	Short bacillus
Spores	None
Gram reaction	Sharply negative
Motility	5 hours, none; 24 hours, none
Gelatin	Liquefaction
Milk	No change
Litmus	Acid; no clot
Plain agar	Heavy growth
Plain broth	Heavy growth
Inulin water	No indol
Peptone water	No indol
Glucose	Acid, no gas
Mannite	No acid, no gas
Salicin	
Saccharose	
Lactose	
Russell's media	
Loeffler media	No liquefaction
Virulence	No virulence for mice

[4] Simon, C., and Wood, W., *Am. J. Med. Sc.*, 1914, cxlvii, 247.

Results of the kind here obtained might possibly be explained as due to a difference in hydrogen ion concentration of the dyes used. It seemed quite unlikely that this explanation was the correct one. The problem had already been studied and it had been found that by changing the hydrogen ion concentration of the agar used for divided gentian violet plates, the normal selective activity of this dye was in no way affected. If the pH of the agar were such that growth of the organisms was at all possible, the dye produced the expected selective result, no matter what the pH was.

In order, however, to check up this fact again, the experiments with *Bacillus X* and *B. anthracis* just described, were repeated, using—instead of the dyes—distilled water adjusted to hydrogen ion concentrations, corresponding to those of gentian violet, acid fuchsin (old Grübler) and acid fuchsin (new Grübler). These experiments were entirely negative, the fluids tested being without any effect on the growth of the organisms. The reverse selective bacteriostatic properties of these dyes are not, therefore, to be explained by variation in hydrogen ion concentration.

3043

Standardization of typhoid vaccine by photometric methods.

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Owing to an inquiry from Dr. Joseph W. Smith, Jr., of the Army Medical School, as to the suitability of the photomètre to estimate the strength of bacterial suspensions, a series of investigations were undertaken. Since the completion of this work, Dr. Smith[1] has published two articles on this general subject. From the result of our own experiments we are in accord with his critical statements in regard to the accuracy of the counts made by

* Membre correspondent de l'Institut Prophylactique, Paris.

[1] Smith, Joseph W., Jr., *Am. J. Pub. Health*, May, 1925, 433; *J. Infect. Dis.*, 1925, xxxvii, 385.

the Wright's method in standardizing bacterial vaccines. Further, we would emphasize the error introduced in laboratories employing women, by their use of the normal erythrocyte count for men, rather than the actual erythrocyte count for the worker. Where the vaccine prepared is an autogenous one the errors are of lesser importance. However, with stock vaccines for administration to several individuals either for treatment or for immunization, the need of at least reasonable precision in standardization is generally recognized.

The present communication is based on the examination of ten bacterial suspensions made from different cultures of the typhoid bacillus, the strength of the suspension being estimated first, by the microscopic counting method of Wright on dry film, second, by the reading for the absorption of light in the photomètre, and third, by the reading for the diffusion of light in the same instrument. For the count, blood slides were made and counted, a dilution of 5,000 million organisms per 1 cc. of vaccine, prepared in accordance with the average of the counts, and later an additional check introduced, by counting the final 5,000 million dilution in similar manner. The stock solution was stored in a special bottle devised to prevent contamination, and permit easy removal of any desired amount, without opening the bottle. The photometer was the photomètre of Vernes, Bricq and Yvon, described in detail elsewhere[2] and devised by them for use in the Vernes flocculation test for syphilis. Curves were plotted from the resulting readings for the absorption and the diffusion of light. We observed a greater variation in the absorption than in the diffusion, pointing to the ability of the former more readily to detect any slight change, and therefore to be considered the more delicate test of the two. As the number of organisms decreased the readings became closer. These lower readings represent the amounts ordinarily used in vaccines, so it would appear that the chance of a significant error in photometric standardization is negligible. The complete paper will appear in the *Journal of Infectious Diseases.*

[2] Baylis, Adelaide B., Sheplar, Adele E., and MacNeal, Ward J., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 1-5.

3044

A relation between experimental hyperthyroidism and barring in poultry.

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[*From the Department of Hygiene, Cornell University Medical College, New York City.*]

When the thyroid secretion of the domestic fowl is augmented by the addition of desiccated thyroid in the diet, or by the injection of thyroxin, changes in the moulting process and in the form, structure and color of new feathers occur, some of which have been described elsewhere.[1] Among these effects, one of the most notable is a modification of the form of certain feathers in the male that have a broad lacy border of naked barbs. The width of this border loses its normal uniformity, becoming narrower at some points than at others, owing to the unusual extension of barbules at these points on to the barbs. The contour of the central barbuled area thus presents a more or less regular series of scallops.

This structural change was first observed in Rhode Island Red males but its significance overlooked because of its relative irregularity. In Brown Leghorn males, however, the regularity of the marking in the hackles suggested a correlation with the pigment bars of barred breeds such as, for instance, the Barred Plymouth Rock. This surmise proved to be correct. When Barred Plymouth Rock males were fed thyroid, their new hackles displayed the same characteristic scalloping, and in this case the scallops corresponded closely with the pigment bars. When male Campines were given injections of thyroxin,[2] hackles, that followed white feathers that were plucked, contrasted sharply with the latter, both in color and structure. Not only was there a quantity of dark pigment, but it was laid down in a pattern in which bars could be readily recognized. And the scallopings characteristic of the feathers of thyroid-fed birds were associated with the pigment bars, as in Barred Plymouth Rocks.

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[1] Torrey, Harry B., and Horning, Benjamin, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 275; *Biol. Bull.*, 1925, xlix, 275; *ibid.*, 365.

[2] Experiments by Mr. Floyd Ruch, a student in my laboratory.

It appears, then, that a fundamental tendency toward rhythmic feather marking was manifested by these birds in two ways: in color pattern and in structure. Under normal conditions, the color type was exhibited by typically barred breeds such as Barred Rocks and Campines (body only). Under the condition of hyperthyroidism experimentally produced, the structural type was demonstrated in feathers with barred color pattern, in pigmented feathers without barred color pattern, and in non-pigmented feathers. The rhythm characteristic of these two types is not referable directly to diurnal metabolic rhythms that appear to determine the fundamental barring which Whitman had described in pigeons.[3]

Whatever the underlying mechanism, its activity has been associated experimentally with the activity of the thyroid.

3045

Further evidence concerning the significance of nuclear inclusions as indicators of a transmissable agent.

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In 1920 Jackson[1] reported the occurrence of what she described as a protozoan infection of the ducts of the salivary glands of guinea pigs. "Round oval, encysted organisms" were found lying in the duct cells of 54 per cent of the guinea pigs examined by this author. Jackson concluded that these structures represented the vegetative cycle of an intracellular protozoan, probably belonging to the group of coccidia.

In 1921 Goodpasture and Talbot[2] confirmed Jackson's findings. These authors found a striking resemblance between the structures described by Jackson and certain protozoan-like cells found by them in the lung, liver and kidney of a two months old baby. The occurrence of cells of this type in infants had been previously

[3] Riddle, O., *Biol. Bull.*, 1908, xiv, 328.

[1] Jackson, L., *J. Infect. Dis.*, 1920, xxvi, 347.

[2] Goodpasture, E. H., and Talbot, F. B., *Am. J. Dis. Child.*, 1921, xxi, 415.

described by several observers, the majority of whom had considered these structures to be protozoan parasites. Goodpasture and Talbot, however, were of the opinion that neither the protozoan-like cells observed in infants, nor the structures in the ducts of the sub-maxillary glands of guinea pigs described by Jackson, were protozoa. They concluded that all these peculiar cells arise through the metamorphosis of certain tissue cells.. They showed that the first evidence of this transformation consisted in the appearance of acidophilic intranuclear inclusions and drew attention to Tyzzer's[3] observations of similar nuclear inclusion bodies occurring in the cells of the cutaneous lesions of varicella.

Von Glahn and Pappenheimer[4] in 1925 reported the occurrence of cells similar to those observed in infants in the viscera of an adult man, and thought that the large inclusion bodies were identical in morphology and staining reactions with the inclusion bodies described by B. Lipschütz,[5] and others, in spontaneous and experimental herpes simplex. Lipschütz has held that these bodies represent a specific reaction of the nucleus to the presence of a virus.

Studies which we have made of the structures described by Jackson have led us to the opinion that they arise by transformation of the epithelial cells and that the large acidophilic bodies are intranuclear inclusions probably of the same general nature as the intranuclear inclusions found in herpes simplex and other virus diseases. It has seemed of interest, therefore, to determine whether a transmissible agent could be demonstrated in the submaxillary glands of guinea pigs in which these structures were present.

We have examined the sub-maxillary glands of 55 full grown guinea pigs and have found the structures described by Jackson in 82 per cent. Some of the glands showed numerous enlarged duct cells with acidophilic intranuclear inclusion bodies, others showed only a few of these cells. In contrast to the findings in old guinea pigs, the submaxillary glands of very young pigs, approximately less than three weeks of age, have showed these cells only rarely, in three instances, out of 43 glands examined. We have inoculated sterile emulsions of the sub-maxillary glands of

[3] Tyzzer, E. E., *Philippine J. Sci.*, 1906, i, 4.

[4] Von Glahn, W. C., and Pappenheimer, A. W., *Am. J. Path.*, 1925, i, 445.

[5] Lipschütz, B., *Arch. Dermat. u. Syph.*, 1921, cxxxiv, 428.

old guinea pigs into the brain, testicle and sub-maxillary gland of young guinea pigs. A piece of each sub-maxillary gland used for inoculation was examined histologically in order to determine the presence of these cells in the material used for injection. In making the emulsions for injection, the glands of several old pigs were usually combined. Young pigs inoculated intracerebrally died in most instances on the fifth or sixth day with meningitic symptoms. The histological examination of the brain of these animals has shown an intense meningitis, the exudate containing chiefly mononuclear cells, in many of which are acidophilic nuclear inclusion bodies. Neither the nuclear inclusion bodies nor the cells containing them are as large as those found in the sub-maxillary glands of the full grown pigs, and the inclusion bodies resemble closely those found in herpes simplex, herpes zoster and varicella. Similar inclusion bodies are found in the testicles and sub-maxillary glands of young guinea pigs inoculated with the sub-maxillary glands of old guinea pigs. In the testicle they occur both in the cells of the tubules and in those of the interstitial tissue; in the sub-maxillary gland only in the cells of the interstitial tissue.

The inoculation of the sub-maxillary glands of old guinea pigs into the brain of old pigs has not produced any meningitic symptoms and the histological examination of the brains of the old pigs has so far been negative. Young rabbits and young rats inoculated intracerebrally with an emulsion of sub-maxillary gland of old guinea pigs have failed to develop symptoms and their brains have been negative on histological examination.

Control inoculations of the sub-maxillary glands of very young pigs (one to two days old), the sub-maxillary glands of old rabbits and the pancreas of an old guinea pig into the brains of young pigs have all been negative.

It has been found difficult to transmit the agent through a series of animals. So far it has been possible to infect only the second animal of a series and this only in four instances; in one instance the second transfer was made from testicle to testicle, in the second, from testicle to brain, and in the third and fourth instances, from brain to testicle.

The sub-maxillary glands of old guinea pigs are still active after being exposed to 50 per cent glycerine for seven days. Heating at 54° centigrade for one hour destroys the activity.

3046

Studies on anaphylaxis with the products of peptic digestion of proteins.

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Experiments were made on anaphylaxis with the digestion products of egg-albumin, recrystallized once. They require completion because the albumin still contained egg-globulin and probably a little ovomucoid. Nevertheless the results seem to leave little doubt as to the formation of sensitizing antigens as a result of the digestion. The digestion products killed, with typical symptoms of anaphylaxis, guinea pigs sensitized several times with 0.1 gm. of these substances, but did not shock animals —with one exception—sensitive to egg-albumen or egg-globulin. Conversely, the animals sensitized to the "peptone" were not definitely sensitive to re-injection with albumin but reacted distinctly to globulin. Acid albumin resulting from peptic digestion of the egg-albumin had practically no effect on the animals sensitized with either albumin or peptone and did not desensitize them against a subsequent injection of the homologous antigen. The nature of the active substance in the digestion products is still to be determined (cleavage or some other change), also the degree of species specificity.

Two peculiarities appeared from the experiments, namely, the fact that the injection of the digestion products had but a very small desensitizing effect (see Walzer and Grove[1]) and, secondly, that a subcutaneous reinjection of the peptone produced a distinct local reaction, hyperemia and edema.

Inconstant effects resulted from injections of horse serum proteins heated for several hours with 10 per cent sulfuric acid on the steam bath.[2] In a number of cases the animals reacted on reinjection of the original protein. It is difficult, however, to exclude the presence of small amounts of the original or slightly changed proteins in the material used.

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[1] Walzer, M., and Grove, E. F., *J. Immunol.*, 1925, x, 483.

[2] Hailer, E., *Arb. a. d. kais. Gesundheitsamt*, 1914, xlvii 527.

3047

On the unity of castor lipase.

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The peculiar activation of castor bean lipase by acids and the changes that this lipase undergoes during germination have attracted the interest of investigators for a long time. The contradictory views on the nature of the activation of castor lipase by acids are partly due to the different character of the two kinds of castor lipase preparations commonly used. Castor lipase in the form of "lipase cream" is in no way altered by water, while the lipase preparation known as "defatted seed" is easily destroyed by it.

In our experiments lipase cream and defatted seed were prepared according to Willstätter and Waldschmidt-Leitz[1]; the lipase estimations were carried out as described by Willstätter,[2] olive oil being used as substrate.

When prepared from resting seed, lipase cream and defatted seed both show that castor lipase has an exceptionally sharp pH optimum around pH 4.7 and no activity in a neutral medium; the lipase is not injured by dilute alkali and its synthesizing power is practically *nil*. If we, however, subject lipase cream to a treatment with aqueous reagents of a pH of 4.7 (dilute HCN, acetate buffer, citrate buffer), it becomes evident that we are dealing now with a lipase which has properties quite different from what they were before such treatment. The former sharp pH optimum at pH 4.7 has disappeared, and castor lipase is now more uniformly active over a much wider range of the pH and active even in a neutral medium. Further, castor lipase thus treated has become easily destructible by dilute alkali and gained considerable synthetic power. It is important to lay stress upon our finding that the characteristic alterations of lipase cream by aqueous reagents of pH 4.7 take place, while the total lipolytic activity is fully pre-

[1] Willstätter, R., and Waldschmidt-Leitz, E., *Ztschr. f. physiol. Chem.*, 1924, cxxxiv, 161.

[2] Sandberg, M., and Brand, E., *J. Biol. Chem.*, 1925, lxiv, 59.

served* These alterations of the lipase, therefore, may be characterized as an "activation" of castor lipase.

If we, however, subject lipase cream to a treatment with aqueous reagents of a pH lower than pH 4.7, or if we treat lipase cream with pepsin solutions, then the activation of castor lipase is complicated by a simultaneous destruction of the enzyme. As far as the characteristic activation of castor lipase by acids is concerned, the action of *watersoluble* acids on lipase cream is not different for different hydrogen ion concentrations, but the same for any pH lower than pH 4.7. It appears to be immaterial, whether HCN, HCl, water soluble fatty acids, amino acids, acid buffer solutions, or pepsin solutions inactivated by heating are used, and whether the acid is formed by autolysis or by pepsin hydrolysis of proteins.

In order to more closely scrutinize the activation of castor lipase, it seemed advisable to use as the enzyme preparation defatted seed instead of lipase cream. We have, however, to recall the fact that the lipolytic activity of defatted seed is easily destroyed by water. If we now let buffer solutions of pH 4.7 act on defatted seed, we expect to find that the lipase is, on the one hand activated by the acid, and on the other hand is partially destroyed by water. But we know already from our experiments with lipase cream that activation of castor lipase and lipase destruction are two separate processes, which may occur simultaneously, but which are not necessarily associated.

An activated lipase powder can be obtained with a destruction of only 35 per cent by treating defatted seed for five minutes with 0.5 N acetate buffer (pH 4.7), centrifuging, washing three times with water for one minute, and drying.[3] Twenty gr. defatted seed of a phyto lipase value of 0.25, containing 501 phyto lipase units, yield 8.6 gr. activated lipase powder of a phyto lipase value of 0.37 containing 321 phyto lipase units (yield 64 per cent).

Activated lipase powder splits fats not only in a neutral medium, but to a certain extent also at an alkaline pH. We find the pH optimum of activated castor lipase around pH 5.6.

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* Such experiments may be carried out under conditions similar to those described by Willstätter and Waldschmidt-Leitz[1] in table 20, p. 211.

[3] Tanaka, Y., *J. Coll. Engin.*, Imp. Univ. Tokyo, 1910, v, 25; *Cf. Chem. Zentralbl.*, 1910, ii, 1637.

The following considerations may offer a tentative explanation of the process concerned in the activation of castor lipase by aqueous reagents of pH 4.7. According to Jacques Loeb, when we have Na gelatinate and add acid, the gelatine salt will give off its Na until the isoelectric point of gelatin is reached, where no more Na is combined with gelatine. Our experiments seem to indicate that castor lipase, as it exists in the resting seed, is combined with a seminal protein on the alkaline side of the isoelectric point (pH 4.7) of that protein, involving the lipolytically active group of the lipase. When we have lipase cream or defatted seed and add acid, castor lipase is given off from its combination, involving the active group of the lipase, until the isoelectric point of the seminal protein is reached where no more lipase is combined. A re-combination of the activated lipase with the seminal protein seems to take place under certain experimental conditions[4] and such re-combination may explain why activated lipase cream is sometimes found to be inactive at a slightly alkaline pH.

It should be noted, however, that when castor lipase is liberated by acid from its inactive combination with a seminal protein, we unfasten only the first of the many ties by which the lipase is linked to the protein material of the seed. Moreover, it becomes evident that the capacity of castor lipase for activation and the primary location of the pH optimum at pH 4.7 are characteristic only of the way in which the lipase is stored in the resting castor bean, but do not constitute a property of the enzyme itself.

As regards the activation of castor lipase during germination, it seems to take place in a way similar to the *in vitro*-activation of the lipase by acid as Connstein[5] pointed out long ago; for it is well known that there occurs in the first stages of germination a distinct shifting of the pH to the acid side. Our knowledge of germination, however, is too limited to understand the details of lipase activation and lipase destruction in the germinating seed.

We should like to mention that activated castor bean lipase cream is especially suitable for use in lecture experiments, when

[4] Willstätter and Waldschmidt-Leitz,[1] experiment 6 on p. 219.

[5] Connstein, W., Hoyer, E., and Wartenberg, H., *Ber. deutsch. chem. Ges.*, 1902, xxxv, 3988.

it is desirable to demonstrate to a large audience within on hour the synthesizing as well as the hydrolyzing power of one and the same enzyme preparation.

3048

L. acidophilus and L. bulgaricus as influenced by surface tension.

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Following the suggestive report of Albus and Holm[1] on the effect of surface tension on lactobacilli, studies were undertaken with some of the same organisms used by them, and we employed their technic, as described in an unpublished paper generously placed at our disposal. Sodium ricinoleate was used as a depressant, for which we are indebted to Dr. W. P. Larson of the University of Minnesota, who very kindly supplied us with a pure product.

Six strains each of *L. bulgaricus* and *L. acidophilus* were tested. While no growth of the former took place in media depressed below 42 dynes, the growth of *L. acidophilus* was abundant at this point and considerably below. *L. acidophilus* was inhibited at 35 dynes as measured by the drop-weight method.

The results, therefore, are in close agreement with those of Albus and Holm, and it appears that surface tension may well be considered an effective criterion for differentiating the very closely allied *L. acidophilus* and *L. bulgaricus*.

[1] Albus, W. R., and Holm, G. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 337-338.

3049

Effect of adrenalin upon blood sugar following ligation of the hepatic artery.*

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[*From the Department of Pathology and the Harry Caplin Pediatric Research Laboratory, the Jewish Hospital of Brooklyn, N. Y.*]

In a previous communication,[1] we demonstrated that exclusion of the arterial supply to the liver by ligation of the hepatic artery and its collateral branches causes death in hypoglycemic convulsions within 15 to 60 hours, depending upon the amount of glycogen previously stored.

The present experiments deal with the effect of adrenalin upon the blood sugar level following this procedure. Five-tenths to 1.5 cc. of 1/1000 solution of adrenalin was injected intravenously at varying periods following ligation of the hepatic artery in dogs. The following observations were made:

1. The degree of hyperglycemia following adrenalin injection varies inversely with the period of time following ligation of the hepatic artery; *i. e.*, the longer the time permitted to elapse after ligation the less the increase in blood sugar.

2. When the animal develops all the manifestations of hypoglycemia, adrenalin no longer influences the blood sugar level.

3. When adrenalin fails to cause an increase in blood sugar, hypoglycemic convulsions and death may be predicted in 2 to 5 hours.

4. When adrenalin had no effect on blood sugar level, the tissues of these animals, examined at death, showed complete absence of glycogen. This is in agreement with the results of the work of Ringer,[2] who showed that in a phlorhizinized diabetic dog, totally depleted of glycogen by shivering, adrenalin does not alter the D:N ratio, through an elimination of extra sugar.

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* With the aid of a grant from the Therapeutic Research Committee of the American Medical Association.

† Harry Caplin Research Fellow.

1 Collens, William S., Shelling, David H., and Byron, Chas. S., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 361.

2 Ringer, A. I., *J. Exp. Med.*, 1910, xii, 105.

5. The blood sugar level does not necessarily indicate the quantity of glycogen stored in the organism. In Dog 20, 1 cc. of adrenalin, intravenously, had no effect upon the blood sugar, although the blood contained 82 mg. sugar per 100 cc. One hour later this animal was in hypoglycemic shock with a blood sugar of 52 mg. Dog No. 15 showed no effect of adrenalin upon the blood sugar, although the blood sugar was 90 mg., and in dog No. 57, 4½ hours before convulsions and death of the animal, the adrenalin had no effect on the blood sugar level at 86 mg. This is important from a clinical standpoint for it would mean that the sugar content of the blood is not an index of the amount of glycogen stored in the tissues.

These observations seem to confirm our previously reported results, that ligation of the hepatic artery causes an abnormally high degree of carbohydrate oxidation with a total depletion of glycogen stores of the body.

3050

A color reaction associated with vitamin D.

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"The chemical relationship between activated cholesterol and the naturally antirachitic substances, such as cod liver oil, yolk of egg, and bone marrow, is one of prime importance in a consideration of the etiology of rickets."[1]

It is definitely established that substances which contain either cholesterol or phytosterol can be made antirachitic by exposure to ultra-violet light. Cholesterol and phytosterol themselves, ordinarily without any curative effect on rickets, can be made antirachitic by irradiation. The criterion for the presence of the antirachitic factor (here called vitamin D) is the "line test" of

[1] Hess, A. F., Weinstock, M., and Sherman, E., *J. Biol. Chem.*, 1926, lxvii, 420.

McCollum and his coworkers.[2] Rachitic animals showing a wide metaphysis free from calcification are fed the substance to be tested in addition to the rickets-producing diet. Deposition of calcium salts, giving a positive line test, shows the presence of vitamin D in the food. This biological test is both expensive and time consuming. A chemical test for vitamin D would save time and effort, and in addition might lead to an understanding of the chemical nature of the vitamin. Hess and Weinstock[3] studied the changes in the ultra-violet light transmission of cholesterol before and after irradiation. Although they obtained a difference, its significance is at present not established. Besides, the method is not generally applicable. The color reactions of substances containing the fat soluble vitamins have been studied by Drummond, Rosenheim and their associates. Of the reagents studied, Rosenheim and Drummond[4] found $AsCl_3$ the most sensitive. They claim that their color test parallels the biological test for the presence of vitamin A.

Harden and Robison[5] state that the purple color given by liver oils when treated with H_2SO_4 can be closely simulated by adding furfural or a substituted furfural to cholesterol or butter. This suggested that the color producing substance in the oil might be related to furfural in behavior. When aniline and HCl are added to a solution containing furfural an intensely red color is obtained. Accordingly, this test was applied to cod liver oil. The aniline reagent was made by adding 1 part conc. HCl to approximately 15 parts aniline. Three cc. of the aniline reagent were added to an equal volume of cod liver oil in a wide test tube. The contents were mixed, heated to boiling with constant shaking, and boiled for about half a minute.

The yellow emulsion turned green, and in a few seconds changed to red. Within a minute or two, the emulsion separated into two layers, the lower one being colored an intense red. On standing the red color deepened. Sometimes the green color reappeared, but further boiling restored the green color. To determine whether the chromogenic substance was destroyed by mild oxidation, 100 cc. of cod liver oil was kept at 100° for an hour

[2] McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1922, li, 41.

[3] Hess, A. F., and Weinstock, M., *J. Biol. Chem.*, 1925, lxiv, 193.

[4] Rosenheim, O., and Drummond, J. C., *Biochem. J.*, 1925, xix, 753.

[5] Harden, A., and Robison, R., *Biochem. J.*, 1923, xvii, 115.

while air was bubbled through. At the end of that time, the tests with $AsCl_3$ and P_2O_5 were negative, as the English investigators reported; but the test with the aniline reagent was more positive than before. The red color developed rapidly even in the cold. This was taken as an indication that some substance other than vitamin A gave the red color in the oxidized oil.

It was hoped that this would prove to be a color test for vitamin D. To test further this hypothesis, a number of substances were irradiated and tested with aniline reagent. The results obtained with the specimens examined are given in the table.

TABLE I.

Substance.	Treatment	Color	Conclusion
Cod liver oil	none	lower layer intensely red	markedly positive
Cod liver oil	mildly ox'zed at 100°	lower layer intensely red	markedly positive
Unsaponifiable fraction of c. l. o.	none	deep red solution	markedly positive
ditto	freed from bulk of cholesterol by CH_3OH	deep red solution	markedly positive
Cottonseed oil	none	lower layer light yellow	negative
Cottonseed oil	mildly ox'zed at 100°	lower layer brown	negative
Cottonseed oil	irrad. 50 min. at 1 ft.	lower layer red	positive
Cottonseed oil	irrad. 65 min. at 1 ft.	lower layer decidedly red	strongly positive
Raw linseed oil	none	lower layer slightly orange	very faintly positive
Raw linseed oil	heated in open dish at low temp. for 15 min.	lower layer orange	faintly positive
Raw linseed oil	heated more strongly for 15 min.	lower layer brown	negative
Raw linseed oil	irrad. 70 min. at 1 ft.	lower layer intensely red	strongly positive
Boiled linseed oil	none	lower layer reddish*	positive?
Olive oil	none	lower layer orange	faintly positive
Olive oil	irrad. 90 min. at 1 ft.	lower layer red	positive
Salt butter	none	lower layer orange	faintly positive
Cocoanut oil	none	lower layer light brown	negative
Cocoanut oil	irrad. 50 min. at 1 ft.	lower layer decidedly red	positive
Cholesterol	none	yellow solution, light brown meniscus	negative
Cholesterol	irrad. 15 min. at 1 ft.	darker soln., reddish meniscus	faintly positive
Cholesterol	irrad. 1 hr. at 1 ft.	red solution	positive
Colorless mineral oil	none	lower layer light yellow	negative
Colorless mineral oil	irrad. 45 min. at 1 ft.	lower layer light brown	negative
Colorless mineral oil	irrad. 2½ hr. at 1 ft.	lower layer brown with an orange tinge	very faintly positive

* Test obscured by dark color of the boiled oil.

Of the particular specimens examined, only cod liver oil and its unsaponified fraction gave a decidedly red color without any preliminary treatment. The unsaponified fraction developed a strong red color immediately even in the cold. Although some of the oils before irradiation gave a reddish yellow, or reddish brown color, they all gave a decidedly and unmistakably red color after irradiation. The only exception was the mineral oil. The red color does not fade; it rather grows stronger on standing and lasts at least several days. Longer heating with the reagent appeared to produce a more marked reaction. In the test with cholesterol itself, the greater the quantity of irradiated cholesterol dissolved in a given volume of reagent, the deeper was the red color obtained. With non-irradiated cholesterol, the color was yellow even at saturation. The irradiated oils reacted negatively with $AsCl_3$.

Apparently these preliminary tests indicate a rough parallelism between the color reaction and the presence of the antirachitic factor. It is emphasized that the substances examined for this reaction have not yet been subjected to the biological test, and it therefore cannot be stated with certainty that substances which contain vitamin D give this reaction, and substances lacking vitamin D do not. However, since usually cod liver oil and its unsaponifiable fraction alone (of the substances studied here) are antirachitic, and since the other substances, excepting the mineral oil, become antirachitic on irradiation, it seems possible that further study may show a close connection, if not an identity, between the antirachitic factor and the chromogenic substance. This relationship is being studied in this laboratory from a number of angles. If such is found to be the case, this color reaction may be utilized for a quantitative estimation, as it is a permanent color.

After the above described work was completed, the author became cognizant of a paper by Bezssonov[6] in which he describes color tests associated with vitamins A and D, obtained with a reagent which he calls phosphomolybdotungstic acid. A comparative study of the two tests is being undertaken.

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[6] Bezssonov, N., *Comptes Rendus*, 1924, clxxix, 572.

3051

The relation of the vagus to auricular paroxysmal tachycardia.

HAROLD L. OTTO. (Introduced by H. C. Jackson).

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A female, age 23, suffering from mitral stenosis and insufficiency of rheumatic etiology, predisposed to spontaneous attacks of auricular paroxysmal tachycardia lasting five to six hours, was observed during two of these spontaneous paroxysms. The injection of adrenalin chloride (1:1000) in a dose of 1 cc. and repeated again in 15 minutes, never failed, whenever she was not previously subjected to the influence of other drugs, to induce a paroxysm of tachycardia, following approximately 15 minutes after the second injection of adrenalin. These induced attacks of tachycardia were indistinguishable from the spontaneous paroxysms in symptoms, rate, duration and electrocardiography. The inference, therefore, is justifiable that the focus or area of origin of the tachycardias must have been identical in both spontaneous and induced paroxysms. This was repeated four times, and in all the result was the same.

When four milligrams of atropin (a vagal paralyzing dose) were administered hypodermically before the exhibition of the adrenalin, a paroxysm of tachycardia failed to appear. This procedure was performed twice. Atropin, therefore, is not a drug which will induce a paroxysm of auricular tachycardia. It seems that the presence of the reflex vagal stimulation induced by adrenalin was a necessary factor in the induction of the paroxysms, and since spontaneous and induced attacks were alike in every respect, it suggests the vagus as a factor in the induction of the spontaneous paroxysms.

These findings are in harmony with those of Lewis[1] and in opposition to those of Galli[2] and others on the subject, to wit "that tachycardial attacks can be provoked in man by atropin, and that withdrawal of vagal influence predisposes to paroxysms of tachycardia."

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[1] Lewis, Thomas, *Heart*, 1909, i, 43.

[2] Galli, G., *Heart*, 1920, vii, 111.

3052

Comparison of digitalis doses in auricular flutter on the auricle and A-V conduction.

JOHN WYCKOFF. (Introduced by H. C. Jackson).

[*From the Cardiographic Laboratory, University and Bellevue Hospital Medical College, New York.*]

Mackenzie,[1] Turnbull,[2] and Lewis[3] showed that auricular flutter passed into fibrillation during the administration of digitalis. Lewis showed that this was due to the action of digitalis upon the auricle. He also pointed out that another useful action of digitalis in auricular flutter, particularly in cases with 2:1 A-V block, was to slow the ventricle by increasing the A-V block.

We have seen 16 cases of auricular flutter with 2:1 A-V block occurring with various etiological and structural types of heart disease. The first 10 of these cases were given digitalis by the body weight method in 4 doses, receiving in the first 24 hours 0.15 cat unit of digitalis per pound. It was noticed that while all except two of these cases responded with a slow ventricular rate, due to increasing A-V block, none of them developed notable changes in the circus movement rates or developed auricular fibrillation until substantially larger doses of digitalis were given.

The last 6 cases have been studied differently. During a control period of one week, it was determined that the flutter was of the permanent type, and that a persistent 2:1 A-V block was present. At least 2 weeks after receiving any digitalis medication a standardized preparation of digitalis leaf (0.1G = 1 cat unit) was given by mouth at intervals never more frequent than every 6 hours. Observations were made before each successive dose, and the amount of the drug was noted which had been taken at the time of the following changes: (a) the first increase of A-V block; (b) the first distinct change in the circus movement rate; (c) the onset of auricular fibrillation; (d) the first sign of any toxic symptoms.

Table I shows the results obtained.

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[1] Mackenzie, James, *Brit. Med. J.*, 1905, i, 759.

[2] Turnbull, H. Hume, *Heart*, 1911-1912, iii, 89.

[3] Lewis, T. L., *Heart*, 1911-1912, iii, 276.

TABLE I.

DIGITALIS NEEDED

No.	Pt.	To Produce A-V Block			To Produce Appreciable Change in Circus Rate			To Produce Auricular Fibrillation			To Produce Nausea		
		In gms. of leaf	In cat units per lb.	Time Days	In gms. of leaf	In cat units per lb.	Time Days	In gms. of leaf	In cat units per lb.	Time Days	In gms. of leaf	In cat units per lb.	Time Days
1	R.	1.3	0.1	1	2.6	0.2	4	2.6	0.2	4	1.9	0.15	2.5
2	H.	1.45	0.11	1	5.2	0.4	12	5.2	0.4	12	5.2	0.4	12.
3	F.	1.65	0.1	1	2.1	0.13	3	2.1	0.13	3	—	—	—
4	N.	2.1	0.1	1	3.3	0.16	5	3.3	0.16	5	—	—	—
5	F.	1.6	0.07	1	2.9	0.15	5	4.1	0.19	9	—	—	—
6	H.	.6	1.046	0.5	3.8	0.28	7	3.8	0.28	7	3.8	0.28	7.

The dose of digitalis necessary to produce auricular fibrillation always exceeded the dose necessary to produce A-V block, and in three of the six cases toxic symptoms appeared before or simultaneous with the onset of fibrillation. In only one of the six cases was there any marked increase in the circus movement rate until the onset of fibrillation. In four of the six cases auricular fibrillation took place only after doses much larger than that usually needed to produce digitalization in other cardiac mechanisms had been given.

3053

Observations on the isolated pyloric segment.

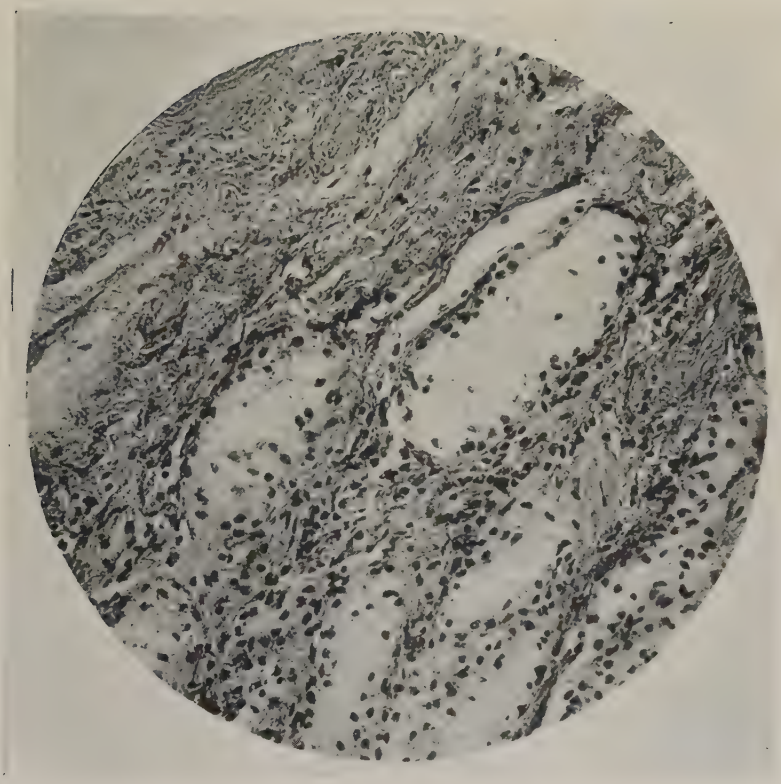
A. LIGHTSTONE. (Introduced by W. H. Barber).

[*From the Department of Experimental Surgery, University and Bellevue Hospital Medical College, New York City, and the Experimental Biological Department of the Pathological Institute of the University of Berlin.*]

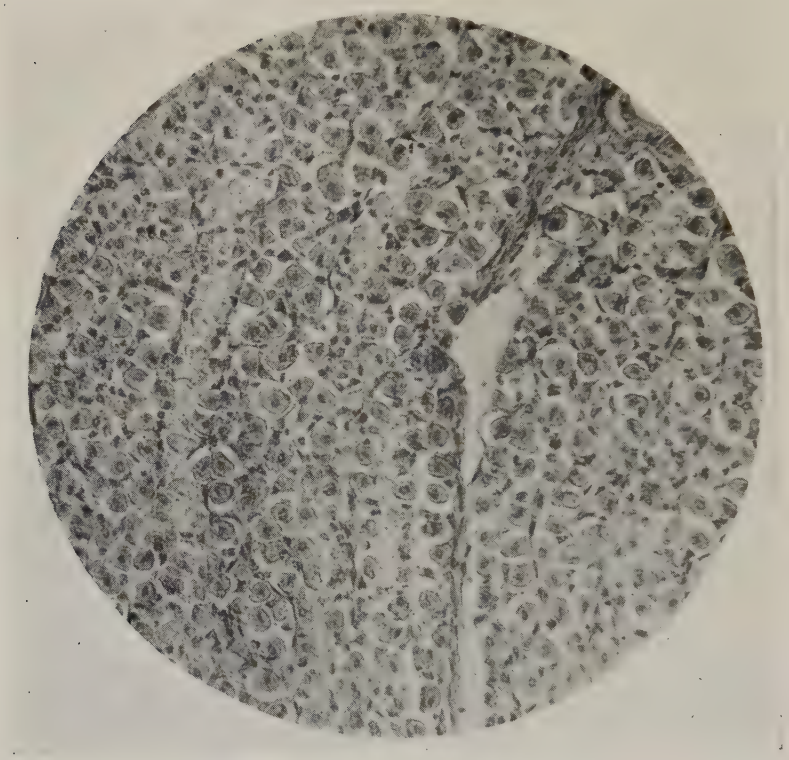
The technic of isolating the pyloric segment from the remaining portion of the stomach in the dog was as follows: Anterior gastroenterostomy was performed between the fundus and jejunum 25 cm. aboral of the pyloric sphincter. The stomach was divided through two planes: first, through the sphincter pylori and second, through the pyloro-fundic region. The pyloric end of the intermediary segment thus created was brought into the wound as a fistula, while the other three cut ends were closed. By this means a closed pyloric pouch of stomach was established, communicating only with the skin surface of the animal. By means of the gastrojejunostomy the animal was fed and kept alive. (See diagram of experiment No. 1.)

Five experiments were carried out according to this technic. Each animal lived from 10 days to 4 months, or long enough to determine (1) the acidity of the pyloric segment under the above experimental conditions, (2) the effects of acetylcholin, pilocarpin, and adrenalin respectively, upon the secretion of the pyloric


Fundus
Pyloric Segment
Fistula
Duodenum
Gastro-enterostomy



Chief cells of the vestibular portion.



Parietal cells of the juxtapyloric mucosa,

segment, (3) the motor function of the residual stomach, and (4) the histology of the fundic and pyloric mucosae.

The conclusions were as follows:

(1) The reaction of the secretion from the pyloric segment was in every instance strongly acid in terms of litmus.

(2) Acetylcholin and pilocarpin produced no appreciable increase in the amount or variation in the acidity of the pyloric secretion. Pilocarpin appeared to be followed by a more mucoid secretion. The adrenalin administration was followed by a distinct decrease in the amount of pyloric secretion.

(3) The residual stomach emptied within 2½ hours through the gastrojejunostomy, in terms of the barium meal and X-ray.

(4) Chief cells predominated in the vestibular portion, the aboral end of the body of the stomach, and parietal cells in the juxtapyloric mucosa.

3054

On the influence of gastric section on gastric secretion.

W. H. BARBER.

[*From the Department of Experimental Surgery, University and Bellevue Hospital Medical College, New York City.*]

Resection of the pyloric end of the stomach is followed by a diminution in the hydrochloric secretion of the stomach. Circumscising the stomach in the pyloro-fundic region through the serous, muscular, and submucus coats of the gastric wall, or the excision of an annular segment from the wall of the stomach in this region, does not appear to be followed by a persistent fall in the gastric acidity, but by such relative decrease in acidity as can be demonstrated after other intra-abdominal operations. When these two procedures are combined, by dividing the pyloric end from the fundic portion of the stomach, and the pyloric part is left in the abdomen (See preceding paper) it appears that the pyloric pouch, thus created, continues to secrete acid.

From these observations, it appears (a) that the pyloric portion is active in secretion (as it is generally agreed to be in motility); (b) that this secretory function continues after the section of vagal or vago-sympathetic nerves incorporated within or running upon the stomach wall (as does also the motor function of the pyloric portion after section of these same nerve fibres).

3055

The toxicity and urinary elimination of various bismuth preparations.

CLIFFORD S. LEONARD.* (Introduced by Lafayette B. Mendel).

[*From the Department of Pharmacology and Toxicology, Yale University, School of Medicine, New Haven, Conn.*]

A method has been devised for the rapid accurate determination of small quantities of bismuth in body-fluids and tissues.

* National Research Fellow in Medicine.

This depends upon the wet combustion of the organic matter followed by colorimetric determination of bismuth as the iodide.

This new method was used for quantitative studies of the urinary elimination and tissue distribution of bismuth after injection of various bismuth preparations. At the same time the toxicity, M.T.D. and kidney pathology were studied. The bismuth preparations used included the soluble sodium potassium bismuth tartrate, sodium bismuth citrate, sodium bismuth thiosulfate, insoluble bismuth oleate, and the insoluble suspension of precipitated bismuth. All bismuth preparations studied exert a toxic action upon the kidney of the rabbit producing necrosis of the tubules.

Twenty-four rabbits were used in the determinations upon which the results here reported are based. The daily urinary bismuth excretion was followed in some cases for three weeks. Dosages were checked when possible upon at least three animals. The intramuscular M.T.D. in the rabbit of the soluble sodium potassium bismuth tartrate is close to 100 mg. per kilo (40 mg. Bi). Twice this dose kills in from 1½ to 5 days. The intramuscular M.T.D. of sodium bismuth thiosulfate is about 150 mg. per kilo (50 mg. Bi). Its effect upon the kidneys is nearly as great as the tartrate, even though here the acidic ion is not nephrotoxic, as is tartrate. Twice the above dose of the thiosulfate kills in from 5 to 6 days. Soluble sodium bismuth citrate is much less toxic. Intramuscular doses of 300 mg. per kilo (200 mg. Bi) of the citrate are just nephropathic, while doses of 125 mg. per kilo (85 mg. Bi) are practically non-toxic. In the case of the insoluble preparations the intramuscular M.T.D. of bismuth oleate is close to 200 mg. Bi per kilo. This heavily necroses of the kidney. 100 mg. Bi per kilo in the form of oleate is tolerated but is still quite nephropathic. An intramuscular dose of 535 mg. per kilo of precipitated bismuth is lethal. 400 mg. per kilo are tolerated, though heavily nephropathic, 85 mg. per kilo are still partially nephropathic. Evidence has been adduced for an additive effect of bismuth and tartrate in producing the toxicity of tartrobismuthates.

The soluble tartrate displays the greatest initial rate of urinary excretion followed by a diminishing rate to death in lethal doses, while sublethal but nephropathic doses show a series of such changes of rate and stoppage of excretion of bismuth. The thiosulfate displays a slightly lower initial rate but, in lethal doses a

similar diminishing rate to death. In sub-lethal dose variation but no sharp stoppage of excretion is shown. The citrate displays a bismuth excretion lower in rate than the tartrate, for the same dose of bismuth, variable, but without sharp stoppage of excretion. The excretion rate of the insoluble bismuth oleate resembles the citrate. The urinary excretion of metallic bismuth is very regular, with the smallest dose of 85 mg. per kilo averaging 1.60 mg. per day. For this dose of bismuth, this is a rate the lowest of all the bismuth preparations studied. All the preparations display a lower rate of urinary excretion and lessened total excretion the larger the dose, and this agrees with the extent of the kidney damage.

In view of the widespread use of thiosulfate as a detoxicant of heavy metal poisoning, the findings with regard to the toxicity and elimination of bismuth after injection of sodium bismuth thiosulfate are of particular interest. Thiosulfate is not a detoxicant of the acute toxicity of bismuth in the rabbit. The rate of excretion of sodium bismuth thiosulfate, while not as great as the soluble tartrate or citrate for the same dose of bismuth, is greater than that of the various insoluble bismuth preparations. Its toxicity is nearly as high as that of soluble sodium potassium bismuth tartrate and far higher than the soluble citrate. On many grounds, it is proposed that the so-called detoxicant action of thiosulfates upon heavy metals may be explained as a mobilization or solvation of insoluble depots which are producing a local pathology in the skin or mucus membranes. The metal when thus mobilized is actually rendered more toxic in its acute effects, such as kidney toxicity, but human therapeutic doses rarely reach a kidney excretion concentration dangerous to normal kidneys even when thus mobilized. The findings as to distribution of bismuth in the tissues will be published later.

(This work will appear in a forthcoming issue of the *Journal of Pharmacology and Experimental Therapeutics.*)

3056

The toxicity and urinary elimination of dipotassium bismuth tartrate.

CLIFFORD S. LEONARD* and JOHN L. O'BRIEN. (Introduced by Lafayette B. Mendel).

[*From the Department of Pharmacology and Toxicology, Yale University, School of Medicine, New Haven, Conn.*]

The intramuscular M.T.D. in the rabbit of dipotassium bismuth tartrate is close to 150 mg. per kilo (75 mg. Bi). The minimal single nephropathic dose is about 100 mg. per kilo (in a two week period). The daily urinary excretion of bismuth after various dosages was followed using the analytical method of Leonard.[1] Nine rabbits were studied in these determinations. The rate of excretion is fairly uniform throughout the survival of the animal after toxic doses and over a two week period in sublethal doses. There is no diminishing rate of excretion such as is shown by the soluble tartrate. There is a lower rate of excretion and lessened total excretion the higher the dose given and this agrees with the extent of the kidney damage. The therapeutic ratio of dipotassium bismuth tartrate is found to be 1/75 using Hopkins[2] M.E.D. against our M.T.D. (This work will appear in a forthcoming issue of the *Journal of Pharmacology and Experimental Therapeutics.*)

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* National Research Fellow in Medicine.

[1] Leonard, C. S., PROC. SOC. EXP. BIOL. AND MED., 1926.

[2] Hopkins, J. D., *J. Am. Med. Assn.*, 1924, lxxxiii, 2087.

3057

Diet and tissue growth. III. The rate of compensatory renal enlargement after unilateral nephrectomy in the rat.

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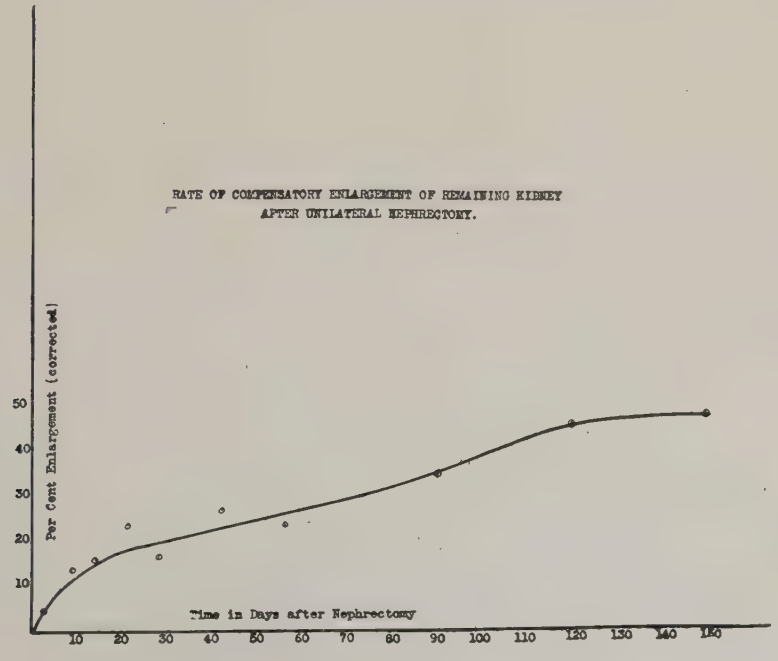
The investigations here reported were carried out incidental to a more extended study of kidney enlargement.

Mature rats were used in order to eliminate as far as possible the factor of increase in size of kidney correlated with somatic growth. The right kidney was removed under ether anesthesia with aseptic technic. At varying intervals, from 3 to 150 days (see chart), after nephrectomy, the rats were killed and the weight of the left kidney compared with that of the control rat. Since there was invariably a small change in the body weight of the rat in the course of the experiment, the final values for the degree of enlargement have been corrected for this factor on the



basis of Donaldson's values. The study here reported is based on observations on 125 animals.

The rats were given *ad libitum* a "synthetic" ration consisting of casein* 18 per cent, raw corn starch 51 per cent, lard 22 per cent, cod liver oil 5 per cent, salts[1] 4 per cent, and in addition 300 mg. dried yeast daily. This diet has been shown repeatedly to be adequate for maintenance and growth.

As may be seen from the chart, there is a rapid increase in the compensatory enlargement of the remaining kidney within the first 3 weeks. At this time the left kidney is about 20 per cent heavier than the control. From the 21st day to the 120th day there is a steady increase at a slower rate (approximately 3 per cent in 10 days) until the enlargement has reached 46 per cent of the control value. From the 120th day to the 150th day our data show no significant increase in size of the remaining kidney, which suggests that the limit of enlargement may have been reached in 120 days.

The enlarged kidneys have shown no gross or microscopic evidence of an anatomical injury.

3058

Chondrodystrophia in chicken embryos.

W. LANDAUER and L. C. DUNN.

[*From the Storrs Agricultural Experiment Station, Storrs, Conn.*]

During the routine examination of chick embryos which had died during incubation, we found in 1923 several embryos exhibiting a striking abnormality resembling the condition known in mammals as *Chondrodystrophia foetais* (Kaufman).* As far as our knowledge of the literature reaches, chondrodystrophia is here reported for the first time in bird embryos.

* The "washed" casein used contained 13 per cent nitrogen. The protein furnished approximately 13 per cent of the dietary calories.

[1] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 557.

* Earlier terms used to designate the same condition are *achondroplasia* (Parrot) and *Micromelia chondromalacica* (Kirchberg-Marchand).

The abnormal embryos were found first in the eggs from one fowl, but subsequently some 50 embryos of the same type have been found among about 4,000 embryos from several strains and varieties of fowls which have been examined. Inheritance may play some part in the causation of this abnormality, although our evidence is conclusive that it is not a simple Mendelian trait. Such embryos (at least those showing this malformation in a recognizable degree) have never hatched. Embryos dying as early as the 12th to 14th day of incubation have shown some of the extreme characters of the chondrodystrophic condition. Most frequently the abnormal embryos die near the end of the incubation period (18th to 20th days), while some have been found still living, but unable to emerge from the shell on the 23rd day of incubation.

FIG. 1.

The external morphology of these embryos, and, as far as our observations go, the histological structure of the cartilage and of the bones as well, resemble very strikingly the characteristics reported for mammalian chondrodystrophia. A typical chondrodystrophic embryo, nineteen days old, is shown in Fig. 1. A comparison of this embryo with a normal embryo of the same age (Fig. 2) shows at once the extraordinary shortening of the legs and the abnormal conformation of the head. In the most extreme cases the legs scarcely protrude beyond the body, the plantar surface is directed towards the body, and the legs are bent towards the body, but are too short to reach each other. All the leg bones are shortened and thickened, but the tibia apparently is affected to a greater degree than the other bones. Even externally

FIG. 2.

the tibia shows in most cases a distinct bending. At this place a triangular plate of bone can often be seen on the inner side of the legs, protruding in a sharp angle towards the outside.

The second striking trait of these embryos is the conformation of the head. The base of the skull apparently is shortened, the parietal and the frontal bones are displaced forward, and the upper jaw shows a marked prognathism. The protruding upper beak is bent downward, giving the embryo a parrot-like appearance. The wings seem to show little or no abnormality. Curvatures of the spinal column have been observed in early chondrodystrophic embryos. In other respects the embryos seem to be normally developed, although the general growth is probably somewhat retarded. Besides the most typical cases we have found a greater number of slighter degrees of this malformation varying to an almost normal appearance. In some of these cases the head seems to be normal but the legs much shortened; in others even the legs do not show a very striking shortening.

Only the bones of the leg have thus far been studied histologically. Although there is considerable variation in the histological picture, yet it shows regularly many of the features found in the bones of chondrodystrophic mammals. The chief departures of the avian from the mammalian type of chondrodystrophia apparently are due to the differences in normal histogenesis in the two classes. The main histological features of the chondrodystrophic leg bones are as follows: The cartilage of the epiphyses shows many irregularities. The perichondrium frequently is thickened. The number of cartilage cells usually is decreased greatly in the peripheral layers of the epiphysial cartilage. The cells are enlarged, and have large cartilage-capsules, in this resembling the cartilage cells of deeper layers of the normal epiphysis. The cells, or if present, the cell-capsules, frequently are contiguous and flatten each other. As a consequence of this fact, little or no matrix is present. The zone of flattened cells, typical for the epiphyses of birds, usually is entirely missing, or only small parts of it are left, in which the arrangement of the rows of cartilage cells is very irregular. An invasion of connective tissue into the epiphysis usually can be seen in the region where the zone of flattened cells normally is situated. The fibrous connective tissue comes mostly from the periost of the inner side of the bone. This periosteal tissue interrupts the longitudinal growth of the bones. In the same region long bow-shaped vessels which in

some cases interrupt the epiphysis for a long distance frequently can be seen. There is probably a causal relationship between these vessels and the invasion of periosteal tissue. The rate of ossification is advanced on the side on which the connective tissue enters the epiphysis. In some cases the invasion of connective tissue from other places than the one stated above has been observed. The formation of columns of calcifying cartilage cells is disturbed. The epiphyses as a whole frequently although not regularly are much increased in size, at times showing a mushroom-like appearance. The periosteal bone reaches far into the epiphysis and surrounds the epiphyseal cartilage like a funnel.

The rate of ossification of the diaphysis is normal or advanced; the ossification itself is increased. The diameter is greater than normal. The tibia shows always, and usually in the same region, a striking bending towards the inner side of the leg. Femur and metatarsus too, frequently are bent, although in a slighter degree. At the place of bending, the trabeculae of the diaphyseal bone show a new arrangement in response to the bending (functional structure). The degree of bending probably depends upon the stage of development at which the formation of the cartilage at first was disturbed, the earlier embryos which we found dead in the shell having a greater bending than the later ones. The bending takes place in a cartilaginous stage of the extremities. The connective tissue seems to enter the epiphysis only in later stages; that is, after the bending of the bones has already taken place. Marrow is present, although frequently reduced in amount. The number of cells in the marrow is increased. Osteoclasts are rare. The number of erythrocytes in the marrow is decreased. A conspicuous alveolate structure fills the place between the marrow cells.

As in human material, the histological appearence of the epiphysial cartilage is very variable, frequently even the two epiphyses of one and the same bone showing considerable differences. The abundance of cartilage matrix in certain epiphyses, the entire absence of the matrix in other epiphyses even of the same embryo, and other observations not mentioned in this preliminary report probably will make it impossible to range the chondrodystrophia of chicken embryos in one of the three classes *(Chondrodystrophia foetalis hypoplastica, hyperplastica,* and *malacica)* which Kaufmann established for human embryos. A complete study of the histology of the skeleton of these embryos is in progress.

3059

The action of atropin, quinin, quinidin, and Ouabain on the fibrillation of the skeletal muscles.

SOMA WEISS.

[*From the Thorndike Memorial Laboratory, Boston City Hospital, and the Department of Physiology of Harvard Medical School, Boston, Mass.*]

The fibrillary contractions of the skeletal muscles of the cat, following the systemic administration of physostigmin, were studied on thirty-three animals. Tracings of the fascicular movements of the pectoralis major muscle were analyzed, and changes in the action current of the pectoralis major, and of the adductor muscles of the hind leg were recorded, according to the method of Forbes and Thacher.[1]

Evidence was presented in a previous communication[2] that the quantitative antagonism between physostigmin and atropin varies in different structures of the body. The antagonism of atropin is complete in the secretory system. It is of interest that atropin sulphate in intravenous doses of 2.5 to 5.0 mg. per kilogram of body weight, prevents or abolishes fibrillation of the skeletal muscles, when the latter is induced by intravenous injection of 0.3 to 0.5 mg. per kilogram of body weight of physostigmin salicylate. These produce marked generalized fibrillation in control animals. Massive doses of atropin do not prevent fibrillation induced by larger doses of physostigmin.

In the case of the skeletal muscles we are dealing, therefore, with a double antagonism, which, above a certain ratio, is complete in favor of the action of physostigmin. The experiments demonstrate a simultaneous drug antagonism with reversed results in different structures of the same animal.

The experiments with atropin suggest that the skeletal muscles contain certain nerve structures, which are parasympathetic in their pharmacological behavior. (Similar observations on the skeletal muscles of man were made recently by Weiss and Kennedy.[3]) Atropin prevents generalized convulsions due to physostigmin.

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[1] Forbes, A., and Thacher, C., *Am. J. Physiol.*, 1920, lii, 409.

[2] Weiss, Soma, *J. Pharm. and Exp. Therap.*, in press.

[3] Weiss, S., and Kennedy, F., *Arch. Neur. and Psych.*, 1924, xi, 543.

Quinin and quinidin prevent or suppress fibrillation induced by physostigmin, the muscular effect of which is not prevented by atropin. The minimal dose of quinidin is smaller than that of quinin, but they are equally effective when the activity is expressed in percentage of the fatal dose. Definite effect was noted on the electromyogram after the intravenous administration of doses as small as 15 per cent of the fatal dose of quinin or quinidin. Neither physostigmin nor atropin has any effect on the clonic convulsions induced by quinin or quinidin.

Voluntary movements of the muscles are maintained after inhibition of the fibrillation with quinin or quinidin, an observation different from that found in the curare-physostigmin antagonism.[4] The quinin and quinidin antagonism is effective before these drugs exert marked stimulating (convulsive dose about sixty per cent of the fatal dose) or depressing effect on the central nervous system.

Quinin and quinidin do not check the effect of physostigmin on the secretory system.

These experiments suggest that quinin and quinidin have a direct action on certain structures in the skeletal muscle substance peripheral to the motor end-plate.

Ouabain, a typical digitalis body, does not influence the fibrillation. This finding is in harmony with the recognized effect of the digitalis bodies on auricular fibrillation, and with the findings of Hatcher and Weiss,[5] that the toxicity of the digitalis and of the quinin group on the cardiac musculature are independent of each other, and that there is no synergism between the two groups.

It is hoped that this study may serve as an additional evidence for the similar physiological and pharmacological behavior of the skeletal and cardiac muscles. The possible therapeautic application of quinin and quinidin in certain tremors requires further study.

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[4] Pal, J., *Centralbl. Physiol.*, 1900, xiv, 255.

[5] Hatcher, R. A., and Weiss, S., unpublished experiments.

3060

A study by new methods of the surfaces of normal and sensitized acid-fast bacteria.

STUART MUDD (with the assistance of Emily B. H. Mudd).

[*From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.*]

Technique and theory of a simple method for study of certain physical chemical properties of the surfaces of bacterial and other cells have been reported.[1] In its present development the method is chiefly useful for examination of cells or other test objects in whose surface lipins are present or suspected, and whose range of size is from that of a bacterium to that of a large white blood cell. It is hoped that other workers will extend and adapt it to their purposes in the study of permeability, cytolysis, action of protective colloids and other problems.

The present report concerns the serum sensitization of acid-fast bacteria. These are tested as follows:

A drop of oil (Tricaprylin, Kahlbaum) and a drop of bacterial suspension are placed upon a carefully cleaned slide. A clean cover slip is placed on them in such a way as to spread the oil into a film under one end of the cover slip, and the watery suspension into a film adjoining the oil. The preparation is then studied under the dark-field microscope. The oil-water boundary surface or interface appears as a bright line, and the bacteria as bright shimmering objects against a dark background. If the preparation has been properly made the oil encroaches slowly on the aqueous phase so that the interface moves across the field and overtakes the sedimented or suspended bacteria and bacterial clumps.

The behavior of the bacteria when overtaken by the interface differs according as their surface is physico-chemically[2] similar to and therefore miscible with the oil or with the water. Normal acid-fast bacteria when touched by the oil are shot violently into the oil phase. If in clumps, the bacteria are dispersed explosively

[1] Mudd, S., and Mudd, E. B. H., *J. Exp. Med.*, 1924, xl, 633, 647; 1926, xliii, 127.

[2] Harkins, W. D., *Colloid Symposium Monographs*, New York, 1925, ii, 162.

by the interfacial stresses and the scattered bacteria suddenly appear in the oil phase. After sensitization with serum the bacteria are no longer easily wet by the oil; they are stable in the interface and if free slide along it; the interface advancing against a sensitized clump attached to the glass bends backward instead of flowing ahead over the bacteria. The clumps are strongly coherent and are comparatively little dispersed by the interfacial stresses. Serum-treated bacteria which show detectable changes in the direction indicated may be said to give a positive interface reaction.

The visible changes effected in the tubercle and other acid-fast bacteria by strong sensitization are thus: (1) that their predominantly oil-miscible (or chemically non-polar) surface has become a water-miscible or chemically polar surface. (2) that the cohesion of the bacteria has been greatly increased. These changes are brought about by high concentrations of normal serum or by high or low concentrations of homologous immune serum. The effect with immune serum is specific, as has been shown both by the titers and by adsorption tests.

Agglutination tests as ordinarily conducted are notoriously unreliable with the acid-fast bacteria. Certain strains, for instance, show little or no agglutination even in potent homologous sera (v. Table II). Additional information of value may be had, however, by a simple modification of the usual macroscopic agglutination procedure. A given amount of an even bacterial sus-

TABLE I.

Comparative tests with three species of cold-blooded "tubercle" bacilli. Little serological relationship shown.

		Fish T. B. Antiserum	Turtle T. B. Antiserum	Frog T. B. Antiserum
Fish T. B. Suspension	Agglutination	80	All neg.	20
	Resuspension	320	All neg.	2½
	Interface	160	5	10
Turtle T. B. Suspension	Agglutination	40	1280*	80
	Resuspension	All neg.	320	10
	Interface	2½∓	80	5
Frog T. B. Suspension	Agglutination	5	10	2560*
	Resuspension	2½	10	2560*
	Interface	2½	5	640

The numbers indicate the titers for the several reactions, *e. g.*, 80 means that the highest dilution of serum producing detectable effect was 1:80.

*Titer not reached.

pension is added to each of the serum dilutions and the tubes are allowed to stand overnight in the ice-box. Agglutination is read in the ordinary way, and the tubes are then centrifuged until clear. The supernatant fluid is poured off and a few drops of salt solution are added to the sediment in each tube. The tubes are arranged in a rack and shaken uniformly until the control shows an even suspension. The organisms which have been treated with the higher concentrations of serum resuspend in flocculi whether or not they showed agglutination by the ordinary procedure. The size and coherence of the flocculi increases up to the highest serum concentrations even where there was a prezone by the usual method. The interface reaction similarly has shown sensitization of the washed bacilli to be maximal after treatment with sera of maximal concentrations, and the interface reaction is positive with inagglutinable strains. The agglutination prezone and the inagglutinability of certain strains are thus due to inhibition of clumping, and not to a failure to bind agglutinins. Agglutinins are bound, but something prevents the bacteria from clumping until they are forcibly brought together in the bottom of the centrifuge tube.

TABLE II.

Three saprophytic strains not definitely differentiated serologically. (3)

		"Mist" Bac. Antiserum	Smega Bac. Antiserum	Pseudotuberculosis Bac. Antiserum
"Mist" Bac. Suspension	Agglutination	20 tr.	10 tr.	20 tr.
	Resuspension	40	40	80
	Interface	40	20	20
Smegma Bac. Suspension	Agglutination	40	20	40
	Resuspension	320	80	80
	Interface	40	40	80
Pseudotuberculosis Bac. Suspension	Agglutination	10 tr.	All neg.	All neg.
	Resuspension	20*	80	320
	Interface	80	80	160

Tr. indicates only a trace of agglutination in any tube of series.

*Titer not reached. Bacteria are sensitized even when ordinary agglutination reaction completely fails to show it.

The interface and the resuspension reactions have both been found to be more reliable detectors of the binding of antibodies by the acid-fast bacteria than the ordinary agglutination procedure.

The immune sera used in this study were kindly furnished us by Dr. J. Fürth[3] and Dr. J. D. Aronson. The fish bacillus in Table I is a new species isolated by Aronson.[4]

3061

A study of the electrical field surrounding heart muscle.

W. H. CRAIB.* (Introduced by Edward P. Carter).

[*From the Cardiographic Laboratory of the Johns Hopkins Hospital and University, Baltimore, Md.*]

In a study of the electrical field surrounding excited cardiac muscle, the writer has applied the present conception of electricity as understood by the electron theory.

It has been possible to advance two sources of experimental evidence, based upon the mathematical theory of a specifically defined electrical conception, from which it seems possible to come to but one conclusion, namely that an element of heart muscle when passing through the stages of excitation, contraction and recovery, exhibits first electrical polarity in one direction for a very limited period of time, and subsequently a reversed polarity for a relatively prolonged period of time.

Before being accepted as applicable to the study in question, the mathematical theory was tested experimentally with complete agreement between experimental and theoretical results.

The above conclusion depends on:

(1) The fact that the measured electrical field surrounding both cold and warm blooded hearts, under certain conditions, can apparently be described accurately in terms of an equation derived as suggested above.

(2) The striking and complete agreement between the deflections theoretically predicted, on the basis of the hypothesis advanced, for varying positions of two electrodes on a strip of cardiac muscle, with those obtained by actual experiment.

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[3] Fürth, J., *J. Immunol.*, in press.

[4] Aronson, J. D., forthcoming publication.

* Loeb Fellow in Medicine, Johns Hopkins University.

(3) The relation between the electrical axis of Einthoven and the cardiac muscle fibres excited at any given instant, as demonstrated by many observers.

It has further been possible to demonstrate that the curve recorded from these simple muscle strips can be shown to be entirely at variance with the previously accepted theory of the spread of the so-called wave of negativity.

3062

The constitutional element in the etiology of pneumonia.

RAYMOND PEARL.

[*From the Institute for Biological Research of the Johns Hopkins University, Baltimore, Md.*]

A detailed genetic and biometric study has been made of a family of 13 brothers and sisters all of whom have had broncho or lobar pneumonia one or more times. One has had it twice, and one has had it three times. Seven of the 13 have died of it. One has tuberculosis of the lungs, and another presents clinical symptoms which make it probable that he also has. There have been in the sibship 87.2 person-years exposure to risk, counting the "infant" deaths to have occurred at 0.3 year, which is probably as fair as any other assumption, it having been shown that the deaths of the first year of life center at 0.3 year. In these 87.2 person-years of exposure occurred 16 cases of pneumonia, or 18 per 100, and 7 deaths, or 8 per 100 person-years exposure. Unfortunately, owing to lack of morbidity data, we cannot make any exact comparison of the case incidence rate of pneumonia in this family with that in the general population. But that it is enormously higher is obvious. Every day experience indicates that nothing like 100 per cent of all persons have pneumonia before reaching the age of 19.

In the case of mortality a more exact approach is possible. If the age-specific mortality rates for pneumonia in the U. S. Registration Area (exclusive of North Carolina) in 1910 are applied to a group of 13 children having the same age distribution as the

TABLE I.

Expected deaths from pneumonia in a family of 13 children, if the age specific death rates of the general population operated, compared with the actual deaths in the family here discussed.

Age	Deaths expected among 13 children	Actual deaths in the family studied
Under 1	0.2	6
1-4	.04	0
5-9	.004	0
10-14	.002	1
15-19	.003	0

sibship under discussion, the results shown in Table 1 are obtained.

It is thus seen that there had occurred in the particular sibship studied, up to the time of this investigation, more deaths from pneumonia than would be expected in twenty-five families of this size on the basis of the usual mortality from this cause.

The material from which the data of this paper are derived is contained in two histories of the Family History Records* of this laboratory. The mode of collection of the records has been described elsewhere.[1] It will suffice to say here that trained field-workers visited the families and collected the information under a variety of critical safeguards to ensure accuracy, and that these records were supplemented by hospital, sanitarium, and health department records.

In the pedigree there are detailed records of 202 blood relatives of the 13 children whose pneumonia presents the problem of the study. The data regarding these 202 relatives were submitted to biometric analysis, with the following results:

It is first of all shown that the explanation of such an extraordinary incidence rate of pneumonia as that observed in this sibship cannot, in any significant degree, be environmental. While the environment surrounding these children was certainly not perfect, quite as certainly it was neither worse than, nor essentially different from, that surrounding thousands of similar sibships in

* Financial aid in the collection of these Family History Records was furnished at different times in the development of the project, by grants from the National Tuberculosis Association, the Russell Sage Foundation, and the Commonwealth Fund. To each of these agencies it is a pleasure to acknowledge my indebtedness.

[1] Pearl, R., Studies in Human Biology. Baltimore (Williams and Wilkins), 1924. *Cf.* Chapt. xii.

TABLE II.
Summary of Results.

		Father's side	*Mother's side*
1.	Case incidence of tuberculosis	6.25 per cent	1.7 per cent
2.	Sex ratio (per cent males)	56.5 " "	53.5 " "
3.	Fertility rate	23.6 " "	15.9 " "
4.	Infant mortality rate	14.0 " "	3.7 " "
5.	Total mortality rate	1.61 " "	1.42 " "
6.	Total poor health rate	7.7 " "	8.5 " "
7.	Total respiratory illness rate	1.3 " "	1.1 " "
8.	Infant respiratory illness rate	0.8 " "	3.9 " "
9.	Childhood respiratory illness rate	1.3 " "	3.2 " "

Baltimore who exhibit a pneumonia rate no different from that of the general population. Furthermore, the time incidence of the pneumonia among these children practically precludes the possibility of simple contagion of the disease from one to another being any significant part of the explanation.

Table II summarizes the similarities and differences between the two unrelated genetic stocks which were combined to form the children of the sibship under discussion.

These results may fairly be stated in the following way: The two stocks which produced the children in the family discussed were, as groups, substantially similar biologically in respect of sex-ratio, of total mortality rate, of total poor health rate, and of total respiratory illness rate. The differences between the groups in these respects are not great enough to suggest that there is, in so far, any significant biological differentiation between them. But, on the other hand, the group on the father's side has an excessive incidence of tuberculosis, a higher fertility rate, a markedly higher infant mortality rate, and lower infant and childhood respiratory morbidity rates, than does the group of people who make up the mother's side of the pedigree. Not all of these differences between the groups are statistically significant, and, in so far as they are not, must be regarded as suggestive rather than probative.

The interpretation of the whole pedigree reached after careful study of all the evidence is that we have, in the father's kinship, a group of people with a definite tendency towards constitutional inferiority of the respiratory system, which manifests itself chiefly in a tendency to breakdown from pulmonary tuberculosis in early adult life. This particular constitutional inferiority is absent in the mother's kinship, but in that group of people there is

definitely manifest a constitutional tendency to generally non-fatal respiratory infections, bronchitis and broncho-pneumonia, in infancy and childhood. When these two constitutional traits were combined, by the mating of the father and mother of the 13 children in the sibship under discussion, there was produced a group of children with extremely low resistance to any sort of respiratory infection, with a consequent 100 per cent incidence of pneumonia in the years of infancy and childhood.

A complete account of this investigation, presenting the detailed evidence, is now passing through the press.

3063

Effect of light of different wave lengths on penetration of 2,-6,-dibromo phenol indophenol into Valonia.*

MATILDA MOLDENHAUER BROOKS.

[*From Division of Pharmacology, Hygienic Laboratory, Washington, D. C.*]

The marine alga, *Valonia,* was placed in solutions of .00035 M concentration of the oxidation-reduction dye, 2,- 6,- dibromo phenol indophenol dissolved in sea water. The dishes containing the plant were then screened with glass screens transmitting various wave lengths from 300 to 700μ and were placed in diffuse daylight before an open window or were kept in darkness. The pH of the solution was 5.4. The temperature was 22° C. with a variation of about 0.5°.

The results show that as the length of the incident light decreases towards the ultra violet end of the spectrum, the amount of dye in the sap increases.

By extrapolating the curves to equilibrium, it was found that the penetration of the dye follows the course of a unimolecular reaction.

By calculations from curves of relative energy distribution in the visible spectrum obtained from figures as given by Luckiesh[1]

* Published by permission of the Surgeon General.

[1] Luckiesh, M., Color and its application. D. Van Nostrand and Co., New York, 1915, p. 20.

for blue sky light and other sources, it was found that the effect of light on the penetration of this oxidation-reduction dye varies, not as a function of the amount of energy, but as a function of the wave length. The details of this experiment will be published elsewhere.

3064

The proof that a hormone is concerned in external pancreatic secretion.

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Direct evidence has been presented by us[1, 2] which proves that a humoral mechanism is in part, at least, concerned in the stimulation of the pancreas to secrete pancreatic juice. Indirect evidence has also been presented[1] which suggests that a hormone is the exciting humoral agent.

We now have evidence which, according to our interpretation, proves unequivocally that a hormone is concerned in the genesis of the external secretion of the pancreas.

This evidence was obtained from two different preparations: In one preparation, a Thiry fistula of the jejunum and a pancreatic transplant was made. In the other preparation, a twelve inch loop of the jejunum was transplanted subcutaneously (4 inches took) and later a pancreatic transplant was made.

When N/10, or N/100 HCl, is applied to the mucosa of the intestinal transplant, the pancreatic transplant secretes copiously. When N/10, or N/120 HCl, is applied to the mucosa of the Thiry fistula, the pancreatic transplant secretes copiously. The latent period of stimulation varies from three to six minutes.

The interesting observation has also been made that fresh olive oil applied to the Thiry fistula does not stimulate the pancreatic

[1] Ivy, A. C., *Proc. Am. Physiol. Soc.*, Cleveland, Dec., 1925.

[2] Ivy, A. C., and Farrell, J. I., *Proc. Am. Physiol. Soc.*, Cleveland, Dec. 1925.

transplant; but if the olive oil is digested with pancreatin, and the neutralized digest (neutralized with Na_2CO_3) applied to the mucosa of the Thiry fistula, the pancreatic transplant is stimulated.

3065

Studies on inorganic salt metabolism. II. The effect of the sudden alteration of the acid-base balance of the diet on dogs.

MARTHA R. JONES. (Introduced by Lafayette B. Mendel).

[*From the Department of Pediatrics, the Stomatological Research Group and the George Williams Hooper Foundation, University of California, San Francisco, Calif.*]

During the course of a study on inorganic salt metabolism in dogs which has been in progress in this laboratory for the past 4 years, it has frequently been observed that convulsions, usually followed by death, occur in the experimental animals after the sudden alteration of the acid-base balance of the diet. The convulsions appear to result from an increase in the acid or alkali content of the diet beyond the limit of tolerance of the animal as well as after the sudden reversal of the dietary reaction. The nutritive condition of the dogs has been excellent, there having been no known dietary fault other than a disproportion of its acid-base balance. The onset of the symptoms has been sudden, the attacks, which are characterized by marked twitching, clonic contractions of the limbs, rapid respiration, gasping for breath and excessive salivation, are of short duration, as a rule. The animals recover quickly and appear to be hungry, eating with relish any food that may be available. From the standpoint of our studies, no significance was at first attached to these observations, the condition being attributed to other causes. It occurred, however, with such frequency, and the convulsive attacks were so similar in the various animals affected, that it seemed there must be some common underlying cause. In order to determine whether or not a disturbance in the acid-base balance of the diet was an etiological factor in producing this condition the following experiment was planned:

Five large adult dogs, 4 females and 1 male, ranging in age from 3 to 8 years, and 2 puppies, 5 months of age were used. All of the animals appeared to be in excellent physical condition. The adult dogs had been used for breeding in the laboratory kennels and had never been known to have had a convulsion. The puppies had been under close observation since birth and appeared to be normal. All of the adult dogs, Nos. 1, 5, 7, 8, and 9, were fed bread, meat and potato, the latter predominating in order to make the diet potentially alkaline. Bitches Nos. 8 and 9, who were sisters, 3 years of age, were given sodium carbonate to further increase the alkalinity of the food mixture, and No. 8, beef suet in addition. Puppies Nos. 107 and 109 had developed rickets spontaneously but had apparently entirely recovered on a diet consisting of bread, milk, meat, butter fat, orange juice, bone ash and hydrochloric acid. No change was made in their food other than an exchange of acid and alkali.

Bitches Nos. 5 and 7, which were 5 and 7 years of age, respectively, were first observed in convulsions about 4 weeks after the bread, meat and potato diet was started. No. 7 became pregnant during this time and had frequent convulsions during the gestation period. At the time of delivery she had a severe attack, went into a coma and was chloroformed 24 hours later. Bitch No. 5 had occasional convulsions during a period of 4 months, but seemed to suffer no permanent ill effects. She also became pregnant and died in a severe attack just before the completion of the gestation period. Dogs Nos. 1, 8, and 9 were not known to have had an attack during the first 5 months on the alkaline diets. The soda was discontinued and an amount of rice equivalent in caloric value to the potato used was substituted for the latter. The animals were watched as closely as possible, but no evidence of convulsions was observed until about the third week after the change in diet. The attacks increased in frequency and severity and finally resulted in the death of all the animals. Puppy No. 107 was suddenly changed from an acid to an alkaline diet by substituting sodium carbonate for hydrochloric acid. Five days later he went into convulsions and died. Puppy No. 109 was gradually changed from an acid to an alkaline diet by first omitting the hydrochloric acid and adding soda in small but gradually increasing quantities. No convulsions were observed during a period of 7 weeks. The diet was then changed to bread, meat and rice in the same proportions used in the other experiments. After

23 days he went ito convulsions which occurred with great frequency until his death 4 days later. Dog No. 1, a large, vigorous male weighing about 80 pounds, proved to be the most resistant of all the animals. His appetite was good and he appeared to be in excellent condition throughout the experimental period. On the day of his death the convulsive seizures started at 10 a. m. and continued without cessation until 3:30 p. m., when he died. His behavior throughout and post mortem findings were typical of all of the animals and will be reported as representative of the group.

Post mortem examinations, which were kindly made by Dr. K. F. Meyer, Director of the Hooper Foundation, showed marked injection of all of the organs, stomach mucosa and subcutaneous tissue, with numerous hemorrhagic areas throughout the body. The heart was greatly enlarged and showed many hemorrhagic areas in myocardium and endocardial lining. The brain was injected and contained small hemorrhages at the base and in the cord, from which a quantity of bloody exudate escaped. The lungs showed terminal pneumonia. Blood failed to coagulate after several hours standing.

No explanation of the phenomena observed will be attempted until histological studies of all of the tissues have been completed. Certain observations on blood and urine which have been made and are now being repeated and extended indicate, however, a profound disturbance in the acid-base balance of the body fluids. On the alkaline diets the H ion concentration of the urine was greatly decreased although the ratio of ammonia nitrogen to total nitrogen was well within normal limits. When the diet was changed from a potentially alkaline to a potentially acid one by the substitution of rice for potato, the increase in acidity was reflected in an increase in the ammonia content of the urine while the H ion concentration was only slightly increased. A few days before death, without any change whatsoever in the diet, the ammonia content of the urine continued to drop until the ammonia nitrogen to total nitrogen ratio was even higher than that observed on the alkaline diet. The reaction of the urine became steadily more alkaline.

These observations indicate a progressive failure of the body mechanism to maintain acid-base equilibrium. The deeply injected organs, greatly distended blood vessels and numerous hemorrhagic areas throughout the body suggest an alteration in the

permeability of the endothelial lining of the vessels. Loeb[1] in his studies on the selective diffusion in living organisms, made on the eggs of the marine fish *Fundulus,* showed that salts accelerate the rate of diffusion of dissociated alkalies and retard the rate of dissociated acids. He also showed they have no retarding influence on the rate of diffusion of non dissoviated acid and perhaps also of non dissociated alkali, and concludes that this probably has some bearing on secretions. It appears that the rate of diffusion of ions through membranes, which is undoubtedly influenced by the concentration, as well as proportions of salts in the body fluids, may have a direct bearing on such conditions as rickets, tetany, so called bronchial tetany, which frequently occurs in rickety babies, hemophilia, certain allergic conditions, spasmophilia, faulty kidney function and many other disorders, the etiology of which is unknown. Experiments are now under way, which we hope will throw more light on the phenomena observed, and will be published in detail, with complete histological studies, at a later date.

3066

The cholesterol content of the hair of the albino rat.

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The data reported herein represent a preliminary study of the lipoids in the hair of the albino rat.

The hair of young normal rats was found to contain 4.5 per cent total lipoids. The total cholesterol content of these lipoids, determined by the digitonin method, was found to be 11.9 per cent, and of this amount 80 per cent consisted of free cholesterol and 20 per cent of combined cholesterol. The amount of lecithin present was calculated from the phosphorus content of the lipoids and amounted to 0.8 per cent of the total lipoids. In a previous communication[1] attention was called to the fact that the lipoids of the human skin contained as much as 20 per cent of total cholesterol. Work is now in progress which will determine the nature of the lipoids in the hair of rachitic rats.

[1] Loeb, J., *J. Gen. Physiol.,* 1922, v, 231.

[1] Eckstein, H. C., and Wile, Udo J., *J. Biol. Chem.,* 1926, lxvii, 59.

3067

Reaction of the urinary bladder in rabbit anaphylaxis.

W. H. MANWARING and H. D. MARINO.

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On intravenous injection of specific foreign protein into hypersensitive guinea pigs, there is a sharp and very marked contraction of the urinary bladder, usually sufficient to increase the intracystine pressure about 40 mm. Hg. Relaxation usually sets in about the third minute, but in fatal anaphylaxis the intracystic pressure rarely falls below 20 mm. Hg. before the death of the animal. This marked, prolonged urinary bladder contraction is consistent with the generally accepted view that smooth muscle contractions play a dominant role in the anaphylactic syndrome in guinea pigs.

In contrast, on intravenous injection of specific foreign protein into hypersensitive rabbits (kymograph control), there is usually no demonstrable increase in the tone of the urinary bladder, even in rapidly fatal anaphylaxis leading to death of the animal in seven to ten minutes. In about a third of the rabbits, a slight contraction is recordable, usually sufficient to increase the intracystic pressure about 2 mm. Hg., with full relaxation in 4 to 7 minutes.

This negative result is inconsistent with the theory that smooth muscle contractions play an appreciable role in rabbit anaphylaxis.

3068

The elimination of certain dyes from the animal organism.

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The observations of Delprat and others[1] that an aqueous solution of rose bengal when injected intravenously into animals is rapidly eliminated through the bile, is not without theoretical significance as well as of practical value. Its behavior in this respect is wholly like that of the bile acids.[2] Rose bengal is not soluble in the ordinary fat solvents and its behavior in the body does not follow the assumption of Mendel and Daniels[3] that substances which are eliminated from the body by way of the bile must be *insoluble in water* and soluble in bile or substances contained therein.

Rose bengal is a highly diffusible substance and it was not found possible to retain it within an ordinary collodion membrane. This property suggested that it might be possible by ligation of the bile duct to force the dye through the kidney. Numerous experiments* which were carried out on both dogs and rabbits showed that after ligation of the bile duct, rose bengal was found present in the blood stream for a period of 48 hours after the injection of the dye. The absence of color in the urines indicated that the dye did not pass through the kidney. Under similar conditions, congo red, a non-diffusible dye which in the normal animal appears in both bile and urine was eliminated in the urine.

That chemical makeup is not without influence upon the path of excretion of the dye from the body is shown by the experiments which are given in Table I. Both dogs and rabbits were

[1] Delprat, G. D., *Archiv. Int. Med.*, 1923, xxxii, 401. Delprat, G. D., Epstein, N. N., and Kerr, W. J., *Arch. Int. Med.*, 1924, xxxiv, 533. Kerr, W. J., Delprat, G. D., Epstein, N. N., and Dunievitz, M., *J. Am. Med. Assn.*, 1925, lxxxv, 942.

[2] Stadelmann, E., *Z. Biol.*, 1896, xxxiv, 1. Foster, M. G., Hooper, C. W., and Whipple, G. H., *J. Biol. Chem.*, 1919, xxxviii, 379.

[3] Mendel, L. B., and Daniels, A. L., *J. Biol. Chem.*, 1912-13, xiii, 71.

* Certain of these experiments were carried out by Mr. J. A. Merrill.

TABLE I.
Mode of Elimination of Certain Dyes.

Dye	No. animals used		Mode of Excretion	
	Dogs	Rabbits	Bile	Urine
Rose Bengal.				
Tetra-iodo-di-chlor-fluoresceïn	2	3	+	
Tetra-iodo-tri-chlor-fluorescein	1	3	+	
Tetra-iodo-tetra-chlor-fluoresceïn	1	2	+	
Phloxin B. B. N.				
Tetra-brom-di-chlor-fluoresceïn	1	2	+	
Phloxin B.				
Tetra-brom-tri-chlor-fluoresceïn		2	+	
Phloxin R. B. N.				
Tetra-brom-tetra-chlor-fluorescein		2	+	
Erythrosine				
Tetra-iodo-fluoresceïn		2	+	+
Di-iodo-fluoresceïn		1	+	+
Eosin				
Tetra-brom-fluorescein		2	+	+
Fluoresceïn				
Tri-chlor-		1	Cannot determine accurately	
Tetra-chlor-		1	Cannot determine accurately	
Hexa-chlor-		1	Cannot determine accurately	

used in the experiments. For the purpose of studying the excretion of the dyes a temporary biliary fistula was made. Injection was made into the venous circulation and urine samples were drawn through a catheter at definite intervals of time.

The experiments indicate that the number of halogens which are present in the dye influence the path whereby the dye leaves the body. Thus, in this series of fluoresceïn dyes it is noted that the dyes which contain six or more halogens, namely the rose bengal series and the phloxin series, are eliminated in the bile, while the dyes which contain not more than four halogens are eliminated both in the bile and in the urine.

Missouri Branch

St. Louis University School of Medicine, March 3, 1926.

3069

Blood sugar observations in late pregnancy complicated by hyperthyroidism.

O. H. SCHWARZ. (Introduced by Leo Loeb).

[*From the Department of Obstetrics, Washington University School of Medicine, St. Louis, Mo.*]

In a comprehensive review of the literature by Rowley[1] concerning the blood sugar figures in pregnancy, the average figures show that there is no appreciable difference from the normal. It is estimated that the blood sugar throughout pregnancy varies from .07 per cent to .11 per cent. Contrary to these facts, Faber[2] recently reported low blood sugar values in late pregnancy, with a lowered renal threshold, a case whose fasting blood sugar was .052 per cent, and whose blood sugar rose with a sugar tolerance test to .15 per cent, with glycosuria and with a subsequent drop of blood sugar to .05 per cent.

I have been observing a case of pregnancy complicated with a moderate hyperthyroidism, with rather unusual blood sugar findings. The patient entered the Barnes Hospital on October 26, 1925, and was discharged on November 30th. She was re-admitted on December 13th and stayed in the hospital ten days. She was again admitted on January 21, 1926, and is in the hospital at the present time. Her basal metabolic rate on the first admission was 30 per cent plus. With rest in bed, at the end of the first hospital stay it was reduced to 17½ per cent plus. During her second admission in December her basal metabolic

[1] Donaldson (quoted by Keene and Hewar), *J. Obst. and Gyn. of British Empire*, 1923, xxx, 345.

[2] Faber, K., *Hospital Hidende*, Copenhagen, 1925, lxviii, 1039.

rate was 29 per cent plus. When she returned in January her basal metabolic rate was 73 per cent plus and on repeating these determinations several times during the present hospital stay it varied between 65 per cent plus and 73 per cent plus. In October the patient weighed 50 kilos and on March 3rd she weighed 58 kilos. She was at term March 2nd and as yet has not delivered. In determining the basal metabolic rate the Hagedorn apparatus was used and the CO_2 output was also determined. The respiratory quotients obtained in this manner showed that in the morning they varied from .75 to .79. Quotients determined later in the afternoon when the blood sugar was low varied between .73 and .76.

On November 23rd, after a comparatively slight rise following the ingestion of sugar, (1¾ gms. per kilo body weight) a very marked drop in blood sugar occurred during the late afternoon, and, at about 4 p. m. the patient was very weak, nervous,

TABLE I.

Date	11-3	11-23	11-27	12-18	2-1	2-17
Hour						
9:00					.075	.08 x
10:00	.086 x	.092	.09 x	.083		
11:00	.217	x	.208	x	.064	.149
12:00	.153	.154	.1012	.186	x	.138
1:00	.118	.148	.086 .079	.156	.197	.079
2:00	.077		.079	.106	.195	.065
3:00	.080	.062 .059		.055	.063	.075
4:00					.052 .062	.082
5:00					.062	
Urine	0	0	0	Tr.	Not ex.	1.32 gm.

x 1¾ gm. sugar per kilo.

and very anxious to return to the hospital ward. The same drop occurred in a similar test on the 18th of December, with similar symptoms. Table I shows the results of these observations. After the experience of November 23rd it was decided to fast the patient during the entire day in order to determine her blood sugar values under such conditions. In consulting Table II, it will be noted that, between the hours of nine and ten o'clock in the morning, the blood sugar was usually about .07 per cent, and in most instances considerably higher, and only on two occasions was it below. It will be seen that in most instances the blood sugar dropped materially from morning to late afternoon, drops as great as 30 milligrams being observed. The determinations were carried out by the Folin-Wu method and prior to January 22nd, the figures are not corrected for the lower values. Using a standard glucose solution (2 cc. = 2 mg.) a correction varying from 4 to 6 mg. should be made, raising the values according to a recent publication by Oser and Karr.[3]

So far as I know, such marked drops during a single day, starting with a normal fasting blood sugar in the morning, have not been previously emphasized. About the same time I made my first observation with the drop following the ingestion of sugar. Marks[4] found that by following the blood sugar curve of rabbits fed for twenty days with thyroid extract, after the injection of glucose (1/6 gm. per kilo) there first appeared a definite rise, with the usual drop during the first and second hours, and at about the time of the second hour a secondary rise appeared. It was found that when this curve was followed further that a marked drop in blood sugar occurred. This was well below .05 per cent, when the animal would go into a state of collapse and could only be brought out of this condition by the injection of more sugar. If no additional sugar was given the animal developed coma and convulsions and died.

Blood sugar curves in cases of marked hyperthyroidism have not shown the results that Marks has shown experimentally. Perhaps the curves in most instances have not been followed sufficiently long. However, this same condition that Marks has reported experimentally has undoubtedly occurred in my case, the pregnancy acting as an additional factor to bring about rapid glycogen depletion in the maternal organism. It will be noted

[3] Oser, B. L., and Karr, W. G., *J. Biol. Chem.*, 1926, lxvii, 319.

[4] Marks, H. P., *J. Physiol.* (British), 1925, lx, 586.

TABLE II.

Date	10-26	10-29	11-3	11-23	11-25	11-28	12-17	12-22	1-21	1-22	1-26	2-1	2-15	2-17	2-25	3-2
Hour																
9:00	.07	.076							.098		.081	.075	.069	.08	.066	
10:00			.086	.092	.09			.08								.080
11:00						.069	.07			.059	.075		.064		.069	
12:00							.067									
1:00						.059		.062			.059		.055		.059	.074
2:00							.052			.045						.070
3:00						.052	.045	.078		.057	.052		.052		.065	
4:00								.078								.070

that this patient, who was first observed late in October, the seventh lunar month of her pregnancy, was just entering the stage of gestation during which the fetus gains excessively in weight. This is particularly well shown by the curves on fetal weight by Donaldson,[1] Streeter,[5] and others.[6] It is also during this time that the fetus stores considerable glycogen. Further, from the seventh month, according to Michel,[7] the fat content of the fetus rises from about 2 per cent to 12 per cent at term. It is well known that the human fetus *in utero* obtains its nutrition chiefly through carbohydrates. There is no definite evidence that fat in any form passes the placental barrier. Therefore, carbohydrates not only furnish the greatest energy to the fetus, but they are used for the storage of glycogen in the fetus as well as being the chief source, if not the only source, from which the fat of the fetus is derived. It will be noted that the last two determinations do not show this marked drop. This, perhaps, suggests that the fetus is now adjusting its metabolism in such a way as to meet the demands of extrauterine life.

With these facts before us one can readily understand how these drops in blood sugar can occur in face of the already existing glycogen depletion as the result of hyperthyroidism and the additional burden of late pregnancy.

3070

The protective action of normal serum against placental extract in vitro.

W. J. DIECKMANN. (Introduced by Leo Loeb).

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In October, 1924, we presented a paper[1] confirming the work of Obata.[2] Obata reported that if normal serum is incubated

5 Streeter, G., Carnegie Inst. of Washington, 1920, xi, Pub. 145.

6 Rowley, W., *Am. J. Obst. and Gyn.*, 1923, xxiii.

7 Michel, *L'Obstetrique*, 1900, v, 252.

1 Dieckmann, W. J., *Proc. Washington University Med. Soc.*, Oct., 1924.

2 Obata, J., *J. Immunol.*, 1919, iv, 3.

with a saline extract of fresh placenta, the latter is no longer toxic to mice if injected intravenously; but that the serum of eclamptic patients obtained during a convulsion or a few minutes thereafter, does not possess this neutralizing power. After the convulsions have ceased, or very early in the puerperium, their sera have regained the full neutralizing power.

Dr. Leo Loeb suggested that we carry out similar work with placental extract *in vitro*. This suggestion is the basis on which the present work was carried out. It was noted by Dold[3] and others[4] that serum would neutralize the toxic action of tissue extracts. Loeb, Fleisher and Tuttle[5] reported on the interaction between blood serum and tissue extract *in vitro* and found that the tissues contain constituents which can combine with a substance in the blood serum and thus lead to the production of a substance inhibiting the coagulation of the blood. Mills[6, 7] obtained from lung extracts a protein called by him "tissue fibrinogen", which had marked coagulation properties. On adding serum to it and then both to the plasma, he obtained the initial marked decrease in clotting time, but on incubating them he found no increase. We found that we could neutralize lung extract just as we did placental extract. His failure was due, we think, to an insufficient amount of serum. Furthermore, we were able to precipitate the active substance from placental extract with the method described by Mills for tissue fibrinogen. The clinical symptoms and postmortem findings after lethal doses of lung and placental extract are identical. Basing our opinion on Obata's work, we had, at the previous presentation, stated that the reaction was specific, but the above work demonstrates that the reaction is a general one.

The following table represents the optimum amounts of each substance as determined by us. We found that citrate plasma gave sharper end points than oxalate or fluoride. Determinations were all made at room temperature and with each unknown serum, a known normal serum was used as control.

Enough placental extract was used to cause complete clotting in 1 minute or less. Usually 0.1 cc. was sufficient.

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[3] Dold, H., *Zeit. f. Immunität.*, 1911, x, 53.

[4] Dold, H., and Ogata, S., *Zeit. f. Immunität.*, 1912, xiii, 667.

[5] Loeb, L., Fleisher, W. S., and Tuttle, S., *J. Biol. Chem.*, 1922, li, 461.

[6] Mills, C. A., *J. Biol. Chem.*, 1921, xlvi, 135.

[7] Mills, C. A., and Matthews, S., *Am. J. Physiol.*, 1922, lx, 193.

	Control	Effect of extract	Serum and extract not incub.	Serum and extract inc. 2 hrs.
Citrate plasma	0.2 ml	0.2 ml	0.2 ml	0.2 ml
2 per cent $CaCl_2$	0.2	0.2	0.2	0.2
Normal saline sol.	0.6	0.5	0.3	0.3
Placental extract		0.1	0.1	0.1
Serum			0.2	0.2
Total volume	1 ml.	1 ml.	1 ml.	1 ml.
Min. and max. clotting time	4-8 min.	45-90 sec.	10-50 sec.	3-8 min.
Ave. clotting time	6 min.	1 min.	30 sec.	4 min.

We found that sera from normal male or female, or from pregnant, parturient, or puerperal women had the power to increase the clotting time if incubated with placental extract. To date, we have had sera from four eclamptics. Two were on our own service and did not neutralize the extract. Two were from other hospitals. One, obtained by me, also did not neutralize. The other had normal detoxifying power. With reference to this sera, my information is that it was taken after the convulsion, but I do not know the interval which elapsed. Furthermore, the patient had only one convulsion and I do not know if the toxemia was a true eclampsia.

Summary: We believe that if normal serum and tissue extract are incubated together for one to two hours and then added to citrate plasma, the clotting time will be decidedly longer than if added immediately; but that eclamptic sera (although our series is small) if taken at the optimum time, will not increase the clotting time, thus indicating that the blood of eclamptic patients either is lacking or does not contain the usual amounts of certain substances always present in normal sera.*

* I wish to acknowledge the assistance of Mr. S. C. Roth in working out the details of the method.

3071

Simplified method of preparation of ovarian hormone, and properties of purified product.

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A detailed study of our former procedure for the preparation of the ovarian hormone from liquor folliculi has shown that several time-consuming steps may be omitted. Tests have indicated that the acetone precipitation of phospholipins may be dispensed with, since the bicarbonate of the liquor folliculi furnishes sufficient alkali to saponify the lipins during the concentration of the alcoholic extract.

Another decided improvement has been effected by a study of the distribution ratios of the hormone and cholesterol between 70 per cent alcohol and petroleum ether. The cholesterol is twenty-nine times as soluble in petroleum ether as in 70 per cent alcohol. A few extractions of the alcoholic solution of the hormone with petroleum ether almost quantitatively remove the cholesterol but only a relatively insignificant fraction of the hormone.

The procedure now advocated is designed to separate the hormone from the soaps and cholesterol. It is the following: The alcoholic extract of fresh liquor folliculi is distilled to dryness and the residue is dissolved in a small amount of water which is transferred to a separatory funnel. The aqueous solution is extracted five times with an equal volume of ether; (in case emulsions form, the addition of a little alkali will aid the separation materially); the ether solutions are combined and washed with a little water, then with dilute acid and again with water. The ether is distilled off and the residue washed with 70 per cent alcohol and petroleum ether into a separatory funnel. If the volume of alcohol used is 100 cc. and the extract represents one liter of liquor folliculi, five washings with 25 cc. of petroleum ether are sufficient to reduce the quantity of cholesterol in the alcohol to less than .01 mg., while the loss of hormone will amount to less than ten per cent. Using this procedure the final product (the 70 per cent alcoholic solution) appears to be a mixture of products

of variable potency, one rat unit ranging from .035 mg. to .075 mg.

Analyses of a purified preparation (.04 mg. = 1 rat unit) by micro methods gave C, 80.8 per cent; H. 10.4 per cent; N, 0.9 per cent; P, 0.00. From the freezing point depression the mean molecular weight was calculated to be 475; based upon the above analyses the smallest gram molecule containing 1 atom of nitrogen must have a weight of about 1500.

3072

The rôle of the sympathetic nervous system in muscle tonus.

JOSEPH C. HINSEY and S. W. RANSON. (Introduced by Leo Loeb).

[*From the Department of Neuro-anatomy, Washington University Medical School, St. Louis, Mo.*]

The left lumbar sympathetic trunk was removed through a median abdominal incision in cats. Autopsy showed the left trunk had been completely removed from the second lumbar to below the brim of the pelvis and that the right trunk was intact. After postoperative periods varying from 50 to 77 days, five of these cats were decerebrated by ligation of the basilar and both carotid arteries. No difference between the sound and the sympathectomized limb could be detected either as to posture or rigidity. Using a simple device, the pressure required to flex the limb was measured in ounces and the time required in minutes. The measurements were repeated several times on each cat and such differences as exist between the two sides, sometimes in favor of the normal side, sometimes against it, disappear when averages of the whole series of measurements are considered.

Three other sympathectomized cats were given tetanus toxin, injecting equal quantities subcutaneously over the femoral trochanter in each hind leg. The degree of rigidity and the abnormal posture which developed in the two limbs were identical. These experiments show that the sympathetic nervous system is not responsible for exaggerated muscle tonus caused by decerebration or the action of tetanus toxin.

The nerve to the vastus internus muscle is being studied to determine the effect of sympthectomy on its unmyelinated fiber content.

3073

The rôle of the dorsal roots in muscle tonus.

S. W. RANSON. (Introduced by Leo Loeb).

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It is hard to see why cutting the sensory fibers for a muscle should abolish its tonus since powerful tonic contraction can be induced from the vestibular labyrinth, from the sensory nerves of the opposite limb and from other sources. It would seem as if afferent impulses from these sources should be able to maintain at least a moderate degree of tonus in the absence of those coming from the muscle itself.

For this reason Frank[1] has suggested that the dorsal roots are important in this connection not so much because they carry sensory fibers from the muscles as because they contain efferent fibers through which tonic impulses are conveyed to the muscles. Such special tonic fibers might be thought of as causing a jellying or increase in viscosity of the contracted muscle thus delaying its relaxation. Frank failed to subject his hypothesis to a thorough experimental test and partly for this reason, and partly because it is in conflict with Bell's law, his theory has received but scant consideration.

The results of our experiments can be made to agree with neither of these theories regarding the origin of tonus. If the long dorsal roots of the sacral and lower lumbar nerves are cut close to the spinal cord and at a distance of from 1 to 1½ inches from the spinal ganglia, the muscles of the corresponding hind limb become atonic for only about 24 hours. After this tonus gradually returns, the extensor muscles usually become markedly tonic and the limb is held in a hyperextended position. There develops considerable resistance to passive flexion. This stiffness usually reaches its maximum about one week after the operation and then gradually subsides during the next two weeks.

Magnus[2] cut extradurally the dorsal roots containing the sensory fibers for the left triceps. The extradural part of the dor-

[1] Frank, E., *Arch. Exp. Path. u. Pharm.*, 1921, xc, 149.

[2] Liljestrand, G., and Magnus, R., *Arch. f. d. ges. Physiol.*, 1919, clxxvi, 168.

sal root is so short that he must have cut within a millimeter or two of the ganglion or possibly through the proximal part of the ganglion. Ten days after the operation he injected equal quantities of tetanus toxin into both triceps. When a small amount of this toxin is injected into an animal the response is sharply localized and takes the form of a continuous tonic shortening of one or more muscles, usually of all the muscles of a single extremity. In Magnus' experiment this tonic shortening developed in the usual manner in the right triceps three days after the injection; but the tetanus toxin had no effect whatever on the deafferented left triceps. He concluded that the muscular rigidity of tetanus is produced reflexly by afferent impulses coming from the affected muscles and passing through spinal cord whose reflex excitability has been increased by the action of the toxin.

We have found, however, that if instead of making the section close to or through the proximal end of the ganglion, the long dorsal roots of the sacral and lower lumbar nerves are cut close to the cord, *i. e.*, at a distance of from 1 to 1½ inches from the ganglia, tetanus develops even earlier in the deafferented than in the normal limb. These experiments show that if one is careful to avoid damaging the spinal ganglia section of the dorsal roots, instead of preventing the development of local tetanus, favors it, so that the muscles of the deafferented limb become rigid even more quickly than do those of the normal side. This demonstrates that the afferent impulses from the affected limb play no essential part in the development of the tonic contraction of tetanus.

Extradural dorsal root section, as practiced by Magnus, probably damages the spinal ganglia and we believe that this damage to the ganglia accounts for the fact that his cats did not develop tetanus in the deafferented muscle. All of our results point toward these ganglia on the dorsal root as playing a very important role in the tonic innervation of the muscles. We have shown, for example, that the local application of nicotine or chloral hydrate to the spinal ganglia interrupts the passage of tonic impulses responsible for decerebrate rigidity. This indicates that these impulses pass through synapses in the ganglia.

Much more work must be done before one would be justified in formulating a general statement as to the part played by the dorsal roots and spinal ganglia in muscle tonus. The diagram illustrates the working hypothesis which we are using as a guide in

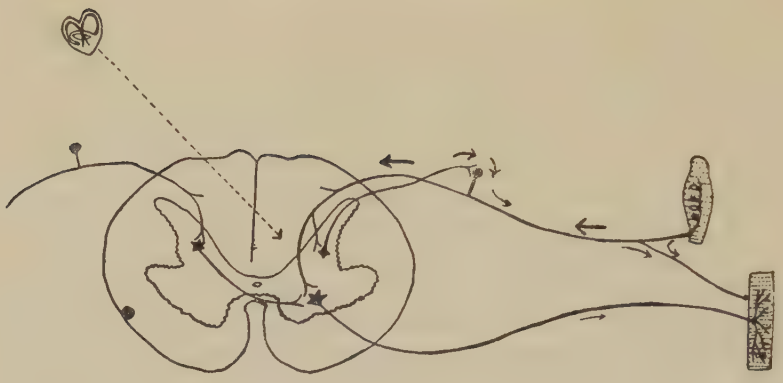

Diagram of possible reflex arcs concerned in muscle tonus.

planning future experiments. It assumes that tonic impulses coming from the spinal cord along the dorsal roots pass through synapses in the spinal banglia and are relayed along the sensory fibers to the muscles. These are assumed to give off side branches with special endings on the muscle fibers. As a necessary corollary we must assume the possibility of axon-reflex-tonus. This would bring the tonic innervation of the muscles into the same category as the vasodilator innervation of the blood vessels.

It is possible that tetanus toxin may act by increasing the excitability of this axon-reflex-arc and this possibility we expect to test by removing the spinal ganglia and allowing the sensory fibers to degenerate before injecting the toxin.

3074

A test for bile salts in urine.

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A search through the text books of physiological chemistry reveals two tests that are used for the detection of bile salts in urine. One is the Pettenkofer reaction which depends on the production of a red coloration when a strong acid, furfural, and

bile salts are mixed. The other, the Hay test, depends on surface tension change due to the bile salts. Because of difficulty in eliminating the urinary pigments, the Pettenkofer test has not been adapted for use as a quantitative test. Recently Meyer[1] has devised a method for quantitating the bile salts in urine dependent on surface tension changes.

The following method can be used as a qualitative test with relatively simple apparatus. (For the quantitation of the bile salts a nephelometer is needed.) It is as follows: Thirty cc. of urine to be tested is mixed with thirty cc. of 95 per cent alcohol. One cc. of 25 per cent trichloracetic acid is added together with a small quantity of charcoal. This mixture is boiled and then filtered. The charcoal remaining on the filter paper is washed with hot alcohol. The combined filtrate is made alkaline with sodium hydroxide and then evaporated to a volume of less than 15 cc. After cooling, the volume is made up to exactly 15 cc. and the fluid is then filtered. To five cc. of this slightly alkaline filtrate is added five cc. of normal sulfuric acid. To another five cc. is added five cc. of distilled water. After standing five minutes the tubes are compared in a beam of strong light. A cloudiness in the tube to which acid has been added indicates the presence of bile salts. The tube to which water was added should be clear.

In this method, as in the Pettenkofer test, the chief difficulty in quantitating the bile salts lies in the elimination of the urinary pigments. Charcoal removes these in part but seldom removes them completely. To overcome as far as possible this interference, we have taken a normal urine, added charcoal and rendered it acid. After boiling it is filtered. This urine is stored after autoclaving, in sterile flasks. In the process of autoclaving the urine becomes rather deeply colored. Dilutions of this sterile urine match fairly closely the tints obtained in the concentrated urine used in the test. The sterile urine solution is diluted until the color intensity of the two solutions is quite similar. A known quantity of sodium glycocholate is added to five cc. of this dilution. Five cc. of normal sulfuric acid is then added and the relative turbidity of the unknown and the standard is determined by reading in the nephelometer.

By this method as little as 2 mg. of sodium glycocholate per

[1] Meyer, E., *Deutsch. Arch. f. Klinsch. Med.*, 1925, cxlvii, 274.

100 cc. of urine may be detected. Jaundiced urines examined have contained up to thirty mg. per 100 cc. Normal urine gives an entirely negative test.

The work with this test has not progressed sufficiently to allow any definite conclusions as to its clinical value. The presence of bilirubin in urine has long been used as an index of liver disease. It is known that certain degrees of liver damage can occur without the appearance of bilirubin in urine. In recent years the search is being made for more delicate methods of detecting liver injury. The bile salts are so peculiarly a product of the liver that they deserve a more careful study than has so far been given them.

3075

The normal and abnormal response of amoeboid cells (amoebocytes of limulus) to stimulation.

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[*From the Department of Pathology, Washington University Medical School and the Marine Biological Laboratory, Woods Hole, Mass.*]

Various considerations suggest very strongly the conclusion that the formation of acid within the amoebocytes is the factor directly responsible for the production of pseudopods and amoeboid movement. It seemed that it might be possible to obtain direct evidence bearing on this problem by allowing substances differing in their chemical and osmotic properties to act on the amoebocytes after the latter have migrated out of the piece of amoebocyte tissue into the surrounding blood plasma of Limulus. However, numerous experiments of this character proved that the amoebocytes respond to stimulation of various kinds with the sending out of normal or abnormal pseudopods and the consecutive movement of the granduloplasm into the latter. The response of these cells does, therefore, not primarily depend upon the character of the external stimulus, but upon the constitution of the cell which is such that it needs to react invariably with certain changes which when taking place in a definite sequence in

a cyclic manner lead to effective amoeboid movement. The amoeboid cell is, therefore, as specifically constituted as the muscle, nerve or gland cell which all react to stimulation of various kinds in their own specific manner.

However, the intensity of the reaction and the character of the movement vary according to the medium which acts on the cells. In general substances which tend to withdraw fluid from the cells and thus increase the viscosity of the protoplasm, call forth the production of thread and sharp tongue pseudopodia, and slow the movement of the exoplasm and granuloplasm, while substances which favor the taking up of fluid on the part of the cells cause the formation of more rounded pseudopods, of drops and balloons, and a more rapid movement of the granuloplasm, which under these conditions tends to fill the balloons more completely. Intermediate reactions of a pendulum character are also observed.

Weak alkali is on the whole more favorable under those conditions to long continued activity of the cells than weak acid. The latter after some time tends to cause a cessation of cell activity, an effect which may be reversible. This result apparently contradicts previous observations made by us which indicated that under experimental conditions differing from those employed in these investigations, acid may favor the outgrowth of the amoebocyte tissue into the surrounding medium. It will be shown in a subsequent publication on which factors the difference in the effects of acid acting upon the cells under different experimental conditions depends.

The most effective medium for the production of multiple thread pseudopodia proved to be a slightly hypertonic solution of $(NH_4)_2CO_3$, while isotonic solutions of this substance tend to call forth the formation of balloon pseudopodia. In general the first effect of the addition of a substance to the amoebocytes consists in the formation of drops and balloon pseudopodia. It seems as if the change of the medium itself acted as a stimulus of so great an intensity that the metabolic changes of a corresponding intensity, produced within the cells, lead to an excess reaction which finds expression in the formation of balloons. When the intensity of the stimulus in the course of time gradually decreases, the viscosity of the cell, or of certain parts of the cell, may increase again and tongue and thread pseudopodia can be produced.

3076

Internal factors in the response of amoebocytes to stimulation.

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In the preceding paper we have shown that the character of the amoeboid response of amoebocytes to various stimuli varies within a certain range in accordance with the character of the solutions with which we surround these cells. However, we found in certain cases that the same kind of solution did not in all experiments produce the same effect, and by varying the conditions of our experiments sufficiently, we could demonstrate that one of the principal causes of such lack of constancy in results consists in the difference in the age of the cells which we use.

By age of the cells we understand the time which elapsed between the beginning of the migration of the cells out of the piece and the testing of the amoebocytes. Young cells are 6 to 12 hours old, cells of medium age are 24 to 36 hours old, and old cells are two or more days old. In general, young cells have a greater tendency to the formation of thread and tongue pseudopodia, while in older cells the tendency to produce balloon and drop pseudopodia is greater. Furthermore, in older cells the tendency to produce under unfavorable conditions pathological formations like courts and pseudofertilization membranes is likewise greater than in young cells. These two factors, favorable or unfavorable character of the medium, and age of the cells, may therefore either mutually intensify, or, on the other hand, neutralize their effects.

To cite some examples: Young cells may in addition to balloons form some tongue and thread pseudopodia in a N/2 NaCl solution, while older cells tend to form exclusively balloons. As we stated in a previous paper, if we add N/1000 HCl in N/2 NaCl to amoebocytes, balloon formation occurs, and if this solution is replaced by a solution of N/1000 NaOH in N/2 NaCl tongue and thread pseudopodia are usually sent out after some

time. In a number of cases we found, however, that even pouring on of alkali may lead merely to the formation of balloons. Whether the one or the other of these two reactions occurs seems to depend upon the age of the cells. It is especially in older cells that the pseudopodia assume the abnormal character of balloons even in alkali. Young cells, on the other hand, may in as unfavorable a solution as an isotonic solution of N/1000 HCl send out at least some tongue pseudopodia, although balloons always predominate in this acid. More frequent is the formation of tongue pseudopodia in an isotonic solution of N/5000 HCl. Also in a solution of NH_4Cl, which in general is not favorable for the formation of tongue pseudopodia, some tongue pseudopodia may appear in very young cells. Likewise, in ammonia, tongue pseudopodia are more frequent in young than in older cells.

Young cells differ, therefore, in certain respects from older cells; the former behave as if their protoplasm was more condensed, more plastic and able to undergo these rhythmic changes in consistency and viscosity on which amoeboid movement seems to depend. On the other hand, the older the cells are the more fluid they have apparently taken up, the more flaccid and flat they tend to be, the less they are able alternately to contract and extend in different parts of their protoplasm, and completely to return to the original contracted state after previous periods of softening. In the course of time the cells have evidently been injured in the medium in which they move, probably as the result of the action of certain substances. Tentatively we may assume that in consequence of cell injury the resynthesis which normally follows the splitting of certain cell constituents remains incomplete, and thus substances which tend to prevent the elimination of fluid from the cell, continue to act within the amoebocytes.

3077

The influence of ammonium salts on the reaction of the protoplasm of amoebocytes.

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We have previously[1] shown that amoebocytes of Limulus stained with neutral red are a favorable object for the study of the penetration of acid and alkali into the living cells. We found that if a N/1000 HCl solution in N/2 NaCl is allowed to act on stained amoebocytes, the acid penetrates within a minute or two into such cells and causes the granules to give off their stain. After this discolorization has taken place, the cells are still able to carry out amoeboid movements, after the acid has been replaced by a N/1000 NaOH solution in N/2 NaCl. The acid penetrated, therefore, into the living amoebocytes. If we surround amoebocytes, previously stained with neutral red with very dilute isotonic or slightly hypertonic solutions of ammonium carbonate, ammonia, or ammonium phosphate, the red color of the granules changes to yellow, indicating that the NH_4OH (NH_3) has penetrated into the cells and affected the granules. Such cells may likewise still be able to undergo amoeboid movement. However, in some cases droplets within the cell may retain the red stain, indicating that the NH_4OH did not penetrate into these droplets or at least was unable to change their acid reaction. If we now replace the ammonium salts or ammonia by isotonic N/1000 HCl, the acid penetrates into these yellow amoebocytes and decolorizes them. As in our previous observations, we noticed that after the granules have given off their stain, the droplets may retain a red color for some time. This suggests that in the droplets the neutral red is not present in the same state as in the granules. In the latter the stain is probably bound chemically or by adsorption to the surface of the granules, while in the droplets it is apparently dissolved in the fluid.

If instead of using these ammonium salts or ammonia, we use a 0.54 N solution of NH_4Cl, which has an acid reaction, the re-

[1] Loeb, Leo, and Gilman, E., *Am. J. Physiol.*, 1924, lxvii, 526.

sults are different. Instead of assuming a yellow color, the cells are decolorized except the droplets which may remain stained red at least for a short time. The cells, into which NH_4Cl has thus penetrated, are still able to carry out amoeboid movements under favorable conditions. NH_4Cl behaves, therefore, in a manner similar to HCl; in both cases the acid entering the cells causes the granules to give off their stain. On the other hand, the amoebocytes differ in their behavior towards NH_4Cl from certain plant cells, into which, according to the observation of Jacobs, NH_4OH (NH_3) penetrates rapidly while the acid remains in the surrounding medium; thus, in an acid solution the interior of the cell assumes an alkaline reaction owing to the much greater power of penetration on the part of ammonia as compared to HCl or other inorganic acids (or of the H ions). Apparently associated with the delicate structure and lability of the amoebocytes is their great permeability to substances to which many other kinds of cells seem to be impermeable or much less permeable.

3078

Presence of a growth stimulating substance in the yolk of incubated hens' eggs.

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It has been shown by Burrows[1] that body cells can grow independently only when they are crowded together into narrow stagnant confines. These conditions are important because this growth depends on the accumulation of growth stimulating substance or substances to a certain concentration. This substance or substances has been called the archusia. The cells cannot re-

[1] Burrows, M. T., and Jorstad, L. H., "On the Source of Vitamin A in Nature" and "On the Source of Vitamin B in Nature." To appear in the *Am. J. Physiol.* in May or June, 1926.

tain it as it is soluble in the circulating fluids of the body. It can be extracted from actively growing tissues, and when added in sufficient quantities to the medium of a tissue culture it causes growth in cells not already containing a sufficiency of it for their growth.

Burrows finds that the blastomeres of the chick and frog embryos cannot form under the same conditions sufficient stimulus for their growth when crowded into a culture medium. For them to grow they must obtain an extra supply of stimulus from other sources. This made it seem certain that the egg must either contain a large quantity of stimulus, or such must be liberated early in its development. The failure for these blastomeres to grow under the same conditions as those suitable for the cells of older embryos, is to be related to the presence of yolk in the blastomeres. This inhibiting action of the yolk has been further associated with the presence in the fat and proteins of the yolk of a lipoid substance which has been called the ergusia. The source of the stimulus for the blastomeres has not been determined. The question arises may it not be contained in the yolk, but its action is overshadowed by the lipoids present there. Carrel and Baker[2] have shown that the lipoid of the egg inhibits growth in the tissue culture. It is possible that a stimulus is present in the yolk and that it diffuses more readily than the lipoid and thus becomes active in the embryonic cells independently of these lipoid substances. Previous work on the addition of yolk to a tissue culture has not shown that it has any stimulating value. In these cases the yolk was added to the medium about the cells while in the embryo it must diffuse through limiting membranes to the cells.

I have recently shown[3] that the growth stimulating substances in embryonic tissue extracts are capable of passing through a colloidin dialysing membrane of a texture sufficiently fine to prevent the passage of proteins in quantities large enough to be recognized by the biuret reaction. It became of interest to determine whether the dialysate of the egg yolk may not be rich in the stimulating substance.

The method used for the estimation of growth is essentially the

[2] Carrel, A., and Baker, L. E., *J. Exp. Med.*, 1925, xlii, 143.

[3] Wright, G. Payling, "On the Dialysability of the Growth Stimulating Substances Contained in Extracts of Embryonic Tissues." To appear in the *J. Exp. Med.*

same as that described by me, the yolk taking the place of the embryo tissue extract in the dialysing vessel. The yolk used in the experiments was taken from eggs which had been incubated for 7 or 8 days. The cells upon which it was tested were emigrant cells from heart fragments of 10 to 11 days incubation. The saline used was the same as that described previously. The estimation of growth was based upon the relative numbers of mitotic figures in experimental and control cultures.

The following figures are averages for experiment and control cultures:

	Experiment.	Control.
Expt. 1 average	127	14
Expt. 2 average	114	14

It would appear from this that at the 7th or 8th day of incubation the yolk contains vigorous growth stimulating substances which, when freed from certain of the yolk constituents, are capable of producing great mitotic activity in artificially cultivated heart cells. It may be of interest in this connection to note that in the course of development the yolk-sac entoderm is separated from the yolk proper by the perilecithal space, a locality free from the droplets of fat of which the greater part of the yolk is composed. This supports the possibility that the growth inhibition is associated with the presence of the yolk fat droplets.

Peking Branch

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3079

The egg-laying capacity of clonorchis sinensis.*

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Evidence presented from our investigation indicates that *egg-laying in Clonorchis sinensis* is neither periodic nor irregular, but is a *continuous process,* beginning as soon as the worm matures and probably continuing until the death of the worm. *Variations in the number of eggs in the stool are due to irregularities in fecal output of the host, to differences in consistency of the stool, and to temporary lodgement of eggs in the bile tracts or gall bladder,* and are not due to actual differences in egg-production per unit of time. Mature worms in recently acquired infections have the same egg productivity as worms that have resided in a host for many months. *In a given species of host egg-production per worm unit of time is constant. There is,* however, *a difference in egg-production in different species of hosts,* apparently independent of the size of the host. For this reason data on the relation of Clonorchis egg-production to the number of worms present in experimental mammals are not directly applicable in human cases of Clonorchis infection. Reckoning from the relation of the average number of eggs per worm per day found in the host's feces to the number of worms found at autopsy of the host immediately after egg-counts have been completed, the *average egg-laying capacity of Clonorchis sinensis in the cat is* 2400; *in the guinea pig, about* 1600; *while in the dog it is estimated at about* 1100.

* Contribution No. 72.

Data are presented to show that *calculations* of Clonorchis worms present in the bile passages, *based on the average number of eggs per worm per diem* in an infection in a particular species of host are more reliable than those based on the average number of eggs per gram of feces (Stoll method). Minimum daily egg-production per worm *per diem* is always sufficient to provide 100 eggs per gram of formed feces, *i. e.*, 1 egg per microscopic slide in decinormal NaOH dilution (Stoll technic), except in very bulky stools such as occur at times in dogs.

This modified Stoll technic, which we have utilized in detecting the presence of Clonorchis ova in experimental animals, is recommended for use in human cases suspected of having Clonorchis infection, and as a check following treatment for clonorchiasis.

3080

Experimental therapy in clonorchis infections.

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Utilizing the method worked out by Faust and Khaw, as presented in the preceding paper, for estimating the number of Clonorchis worms present in the bile passages of laboratory animals, on the basis of the average daily egg count from weighed samples of stools, the authors have tested the effect of gentian violet and mercurochrome on cats and dogs. Both mercurochrome and gentian violet, administered orally in the form of salol coated pills, have a direct effect on egg-production in Clonorchis, in that they greatly accelerate the speed of production. Under these conditions eggs are immature when laid and are frequently imperfect and non-viable.

This phenomenon of hyperproductivity of Clonorchis ova after therapeusis is not directly dependent on the clonorchicidal effect

* Contribution No. 73.

of the dye. In the mercurochrome series living worms with empty uteri were recovered some days after hyperproduction of ova had been observed, in numbers consistent with the previous worm estimate. This appears to refute the idea that the immature eggs in the feces are due to the disintegration of dead worms.

Mercurochrome in doses lethal to the host has no apparent clonorchicidal value. On the other hand tolerated doses of gentian violet will apparently cause the death of all Clonorchis worms in those parts of the bile passages which can be reached by the dye. In light infections this may constitute the entire number of worms (complete cures), as determined by autopsy findings. In heavy infections eradication up to 90 per cent or more of the worms can be secured whenever the host is able to tolerate intensive treatment.

Oral administration of gentian violet in small doses requires prolonged treatment to secure similar results. Doses of gentian violet (Grübler) administered *peros* to cats and dogs, within the limit of tolerance as determined by continued good appetite, absence of nausea or emesis, and sustained or increased body weight, consist of not more than 35 mg. *per diem* per kilo of body weight are toxic to the host and cause death if repeated frequently.

The effect of gentian violet as a clonorchicidal agent is probably indirect. The continued excretion of the dye into the bile passages either from intensive or from prolonged treatment ultimately provides a concentration of the dye in the milieu of the fluke sufficient to cause its death. When the egg count has been reduced to zero a few worms may still be present in bile passages or outpocketings of the bile tracts which are reached with difficulty by the dye. A single maximum tolerated dose will frequently kill these remaining worms, resulting in complete cure.

The results obtained in the administration of gentian violet in experimental animals indicate the possible advantage of utilizing gentian violet therapy in human clonorchiasis.

3081

Further studies of arterial hypertension.

JAMES R. CASH.

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In a previous paper[1] the results of experiments were reported which showed that, in dogs, a marked rise of arterial blood pressure followed reductions of renal substance varying from 50 to 85 per cent. The injury to the kidney was, in most cases, caused by ligation of one renal artery alone, or combined with ligation of one or more branches of the opposite renal artery. When, however, the corresponding reduction of renal tissue was accomplished by unilateral nephrectomy, no rise in blood pressure occurred. In none of these experiments could evidence of renal insufficiency be obtained by examination of the blood for retention of urinary constituents; nor was there any striking alteration of the ability of the remaining functioning kidney-tissue to excrete phenolsulphonphthalein.

The suggestion, therefore, rises that the rise in blood pressure was occasioned by damaged renal tissue left within the body and,, if this is true, it becomes necessary to determine whether this pressor action is characteristic for renal tissue alone under these conditions or is common to all tissue undergoing similar degeneration.

The following five groups of experiments summarized in tabular form were performed in the attempt to throw light upon these two points.

The blood pressure was taken without anesthesia by the auscultotory method, using a regular Bowle's stethoscope and the specially constructed cuff of the Koll's apparatus.[2] This method does not appear as satisfactory as that devised by Kolls, but as the table shows, it seems to be relatively accurate. The Kolls method has so far been impracticable in China because of the deleterious effect of the climate on the thin rubber bulb.

From 10 to 30 blood pressure determinations were made before

1 Cash, J. R., *J. H. H. Bull.*, 1924, xxxv, 168.

2 Kolls, A. C., *J. Pharm. Exp. Ther.*, 1920, xv, 443.

TABLE I—SUMMARY.

Type of experiment.	No. exp'ts	Average blood pressure.		
		Before op'n	After operation.	
		Indirect	Indirect	Direct.
Bilateral ligation of renal arteries	5	140/70	200/136	162
Bilateral nephrectomy	2	126/70	138/64	93
Bilateral ligation of renal artery, vein, and ureter	*2	140/70	123/65	95
	**1	175/80	240/160	186
Splenic infarction	3	127/68	128/75	108
Splenic infarction combined with bilateral nephrectomy	4	135/70	125/68	72

*Renal capsules intact.
**One renal capsule torn. Escape of autolyzed kidney into peritoneal cavity.

any experimental procedures were undertaken. The blood pressure was measured both indirectly and directly, 24 to 48 hours after the operations were performed. There could be no question as to the accuracy of the direct determinations, which were done by cannulating the femoral artery and making a continuous graphic record for 5 minutes. Most of the dogs were too ill to take any notice of this procedure, and in those where only splenic infarction was done 1 per cent novocaine was used in isolating the artery.

After bilateral ligation of the renal arteries, there occurred in each of the five dogs a rise of systolic pressure, varying from 60 to 75 mm., and of diastolic pressure varying from 40 to 85 mm. of mercury.

In the second group of experiments it was found that no rise in pressure followed bilateral nephrectomy.

Complete isolation and destruction of both kidneys by separate ligation of artery, vein and ureter followed by stripping away the peritoneum covering of the kidney and mass-ligature of the renal pedicle caused no elevation of blood pressure. In one experiment where the capsule of one kidney was torn and the autolyzed renal tissue allowed to escape into the peritoneal cavity, a marked rise of both systolic and diastolic pressure occurred.

Infarction of the spleen by ligation of its arteries and by injection of insoluble starch in nephrectomized and non-nephrectomized dogs caused no rise of blood pressure.

These experiments seem to indicate clearly that renal tissue undergoing destruction within the body contains a substance which causes marked elevation of both systolic and diastolic pressures if allowed to escape into the general circulation. This pressor substance cannot be demonstrated in the spleen under the same conditions and thus far is apparently specific for the kidney.

3082

The development of flagellates in Chinese sandflies (phlebotomus) fed on hamsters infected with Leishmania donovani.

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Sandflies (Phlebotomus) have assumed a particularly important position in the study of the leishmaniases since Knowles, Napier and Smith[1] reported the appearance of herpetomonad flagellates in a large proportion of *Phlebotomus argentipes* fed on kala azar patients in Calcutta.

The present paper is a report of certain phases of studies undertaken on the sandflies of North China as possible transmitting agents of kala azar. Three species of Phlebotomus are known to us, namely, *Phlebotomus major* var. *chinensis* Newstead, and two unidentified species which we have designated *Phlebotomus "B"* and *"C"*. *Phlebotomus "B"* is apparently the unnamed species mentioned by Newstead.[2] These three species occur in markedly variable proportions in several regions near Peking and Hsüchowfu, Kiangsu.

In these studies (1) sandflies captured in houses of kala azar patients and elsewhere have been examined for flagellates. (2)

* Assisted by grants from the China Medical Board of the Rockefeller Foundation.

1 Knowles, R., Napier, L. E., and Smith, R. O. A., *Ind. Med. Gaz.*, 1924, lix, 593.

2 Newstead, R., *Bull. Ent. Res.*, 1916-17, vii, 191.

Sandflies reared in the laboratory have been fed on kala azar patients and on hamsters heavily infected with *Leishmania donovani*. In the attempt to transmit kala azar these sandflies have been refed upon tested negative hamsters, and a certain number have been inoculated into other hamsters. As many as possible of such sandflies were examined for the presence of flagellates. A total of over 250 hamsters used in these transmission studies were all negative by liver puncture from 81 to 137 days after the experiments, but final results will be reported from autopsy findings. It is desired to report at this itme (1) a technique of rearing sandflies and of feeding them on hamsters, with particular reference to the feeding of these insects a second time, a feature hitherto little studied, and (2) the results of examination for herpetomonad flagellates.

Technique. The method of rearing sandflies, which will be reported in detail in a separate paper, is a modification of the methods of Waterston[3] and Smith.[4] The breeding vessels are porous earthen pots with a thin lining of plaster of Paris. Over the top of the pot is tied a cover of bolting cloth pierced by a glass tube for the entry of the sandflies. The insects in the pot may readily be seen through the bolting cloth against the white plaster. The pot is set in a dish to which is added sufficient water to keep the plaster lining moist. Males and engorged females are introduced through the glass tube. Oviposition takes place on the plaster walls. The bolting cloth cover is removed as soon as the adults have died, and an earthen dish is inverted over the pot. Crushed feces of negative hamsters are supplied a few days before the estimated hatching of the eggs. The feces become somewhat moldy, but although a few of the very young larvae become entangled in the *fungus mycelia* the presence of the mold in our experience is an advantage rather than otherwise. After pupae are observed the bolting cloth cover is replaced. To obtain individual specimens or to transfer the adults from one vessel to another, they are first released in a cubical cage, provided with cloth sleeve, of the type commonly used for feeding winged insects, except that the cage is made of bolting cloth (No. 50, *i. e.*, fifty meshes to the inch) which permits the sandflies to be readily seen, with no possibility of their escape through the meshes.

[3] Waterston, J., *Ann. Trop. Med. and Parasit.*, 1922, xvi, 69.

[4] Smith, R. O. A., *Ind. J. Med. Res.*, 1925, xii, 741.

They may be caught against the walls of the cage in a small glass tube. This tube, which fits over the glass entry tube of the pots, is provided with a glass plunger by means of which the sandflies may be induced to run quickly through the tube into the pot.

Feeding Sandflies. The method of feeding sandflies which we have found most successful is the use of a feeding cage essentially the same as that used by Wolbach, Todd and Palfrey[5] for feeding lice. This is made from a metal ointment box from which the centers of cover and bottom have been cut out. Bolting cloth (No. 50) is fastened over the bottom and another piece which serves as the cover is held fast when the metal cover is pressed into place. The edge of the box is bound with adhesive tape. The sandflies are introduced through a glass tube in the bolting cloth cover similar to that of the breeding pot. The Wolbach feeding cage is bound with adhesive tape to the forearm of a patient, and may safely be left over night. In the case of hamsters, the latter are made fast against a coarse screen by fine leather thongs around the four legs, and the feeding cage is applied to the closely clipped belly. Our routine was to leave the Wolbach cages on the patients over night, but for only two hours at a time on the hamsters. Occasionally a hamster in its wire cage was kept over night in the large bolting cloth cage into which sandflies had been released. The use of the Wolbach cage, however, was much more successful as well as more convenient.

EXPERIMENTAL WORK.

Material. Phlebotomus "C" apparently has two or more broods per season. Specimens were taken from the middle of June to the end of September. An abundant second generation, the progeny of wild sandflies, was reared in the laboratory during the summer. *P. major* var. *chinensis,* on the other hand, seems to be single-brooded, being found chiefly during June and the first part of July. Only a small fraction of these sandflies in the breeding pots completed their cycle during the summer and as a result laboratory-bred material of this species has been scanty. The experiments here reported were performed at Hsüchowfu during the season of 1925.

[5] Wolbach, S. B., Todd, J. L., and Palfrey, F. W., *Etiology and Pathology of Typhus,* Cambridge, 1922.

Wild Phlebotomus "C". We have examined a total of 378 females and 48 males captured in two villages near Hsüchowfu from June 16th to September 30th. The majority of these were from kala azar houses. About half the specimens were living when taken for dissection, and the remainder, though dead, were still suitable for examination. Smears were negative for flagellates in all cases.

Feeding Experiments with Laboratory-bred Phlebotomus "C". The sandflies were allowed to engorge on kala azar patients or on heavily positive hamsters and were then allowed to feed on tested negative hamsters. The results of the first feeding on the infected host may be summarized as fractions in which the numerator is the number of females which actually sucked blood, and the denominator the total number of females given the opportunity to feed: Females fed on kala azar patients, 168/218; on positive striped hamsters 115/193; on positive giant hamsters 200/302; total 483/713.

The engorged females along with males were transferred to autoclaved breeding pots. Those which died were removed as promptly as possible and examined microscopically. The survivors after periods varying from one to thirteen days were given the opportunity to reengorge on tested negative striped hamsters. Of 286 thus given the opportunity, 140 are known to have sucked blood. Most of these refeedings took place four to seven days after the first feed.

Microscopic Examination. Of the 483 females of *Phlebotomus "C"* originally fed on kala azar patients or infected hamsters, 373 were dissected and examined microscopically, either before or after opportunity to refeed. A number were alive when taken for dissection. The remainder were dissected as soon after death as possible, usually within 24 hours, and the tissues were for the most part quite fresh. Herpetomonad flagellates were found in seven of the 373, either on fresh examination or in stained smears. The other 366 were entirely negative for flagellates and most of these were negative for bacteria or other organisms. These dissections were made from one to fourteen days after the potentially infective feed, the majority being after from three to ten days. The seven positive for flagellates were distributed as follows: four on the third day, and one each on the fourth, fifth and eighth days.

The 373 females examined had fed originally as follows: On kala azar patients, 141; on positive striped hamsters, 75; and on positive giant hamsters, 157. Of the seven containing flagellates, six had fed on giant hamsters and one on a striped hamster, while those fed on patients yielded none with flagellates.

Wild Phlebotomus major var. chinensis. Smears were made from sandflies captured in and near Hsüchowfu as follows: 16 females, 44 males, 12 sex not recorded. Microscopic examination was negative for flagellates in all cases.

Feeding Experiments with Laboratory-bred P. major var. chinensis. A total of 51 females engorged on either patients or positive hamsters out of 149 given the opportunity, as follows: Kala azar patients, 15/58; positive striped hamsters, 0/3; positive giant hamsters, 36/88. Of these 51 females, 31 were given the opportunity to refeed on tested negative striped hamsters. Seven are known to have refed.

Smears were made from 45 of these 51 females. Herpetomonad flagellates, in some cases in great numbers, were found in 29 out of 34, or 85.3 per cent of those which had fed on positive giant hamsters, in marked contrast with the very small proportion, 3.3 per cent, which developed flagellates in the case of *Phlebotomus "C"*. These organisms occurred chiefly in the oesophagus or anterior portion of the mid-intestine, and occasionally in the oesophageal diverticulum. Eleven of those which had fed on kala azar patients were examined and were all negative for flagellates. The flagellate-containing sandflies were dissected from four to eight days after the infective feed.

Phlebotomus "B". This species is rare in the Hsüchowfu region, intensive collecting yielding only a few specimens in nearby villages. While this sandfly is known to feed on man in nature, our attempts to feed over 70 females on man and hamsters were entirely unsuccessful. Smears of 12 females and 9 males were negative for flagellates.

The herpetomonads which develop in sandflies fed on kala azar hosts have not as yet been definitely identified as *Leishmania donovani* by either the Calcutta workers or ourselves, though all evidence at present available renders such identity probable.

3083

Soy sauce as a stimulative agent in the development of beriberi in pigeons.

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Miyadera and Tsuji found that on a diet deficient in vitamin B there is a diminished gastric and intestinal secretion, but the secretory function is not lost and can be restored at once to a normal level if a suitable stimulus is present in the food. Abel and Kubota found that histamine is one of its constituents which accounts for the stimulating action of the soy sauce on the intestinal plain muscle, and that it also plays an important role in digestion as a dilating agent for the capillaries of the gastric and intestinal mucosa. Recently Kubota found that *in vitro* peptic and tryptic digestion of foods is stimulated from 4 to 8 times by soy sauce. Soy sauce possesses also a strong amylolytic function.

Funk reports that spontaneous cures of beri beri in rice-pigeons occur if nothing but water is given. It seems, therefore, probable, that beri beri in pigeons may be produced by some toxin absorbed from the intestinal tract. Soy sauce, in stimulating intestinal digestion and absorption, may also stimulate the production and absorption of this toxic substance.

In order to investigate this question, seven pigeons were fed on polished rice and water, the latter containing five per cent of the Pekingese soy sauce. Five control pigeons were fed on polished rice and pure water. Within one month five out of the experimental pigeons developed beri beri, and of the controls none. The pigeons fed on polished rice and soy sauce and water frequently showed attempts to disgorge the rice out of their crops. One of them, while under observation, died on the 6th day in a few minutes with the symptoms of an acute intoxication, with unsuccessful attempts to disgorge the food from his crop (the latter being only half filled). In one pigeon a typical spastic form of beri beri was observed on the 20th day. In three other pigeons a paralytic form of beri beri developed on the 14th, 27th and 29th day. In one of them this form changed in a few hours into a spastic-paralytic form, although yeast feedings had been started in the

meanwhile. In one of these three pigeons bloody feces were observed.

These data show that soy sauce in some way stimulates the development of beri beri in pigeons, because none of the controls developed beri beri within the same period, although one month is usually sufficiently long for the occurrence of the disease. The case in which beri beri appeared strikingly early (in 6 days) is of particular interest.

3084

The relation of virulence to the pneumococcidal activity of normal rabbit serum-leucocyte mixtures.

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Previous work by Robertson and Sia[1] has shown that the blood (serum and leucocytes) of certain pneumococcus-resistant animals possessed destructive properties for pneumococci not found in the blood of susceptible animals. This suggests that the natural immunity of pneumococcus infection depends chiefly, if not entirely, on the pneumococcidal activity of the blood. In their studies pneumococci of high virulence only were employed, and the animals tested represented well marked examples of natural immunity and susceptibility, the dog and cat on one hand, and the rabbit and guinea pig on the other. It is a commonly observed fact that within a single species of any of the usual laboratory animals there occur wide variations in susceptibility toward different strains of disease-producing pneumococci, and that among the so-called susceptible animals such variations may be extreme.

Experiments were undertaken with the purpose of determining whether the immunity shown by a relatively susceptible animal, as the rabbit, against certain strains of pneumococci was asso-

[1] Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, xxxix, 219.

ciated with demonstrable pneumococcus-destroying powers in the blood. The technique employed was the same as that used by the above mentioned workers. This consists, briefly, in seeding varying quantities of pneumococci into mixtures of rabbit serum and leucocytes contained in small tubes, which are then sealed with paraffined corks and attached to an apparatus inside the incubator whereby constant agitation is carried on during incubation.

It was found that the rabbit serum-leucocyte mixtures possessed the power to kill avirulent pneumococci in relatively large numbers but failed to inhibit the growth of virulent organisms even in minute quantities. The results of numerous experiments in which all three types of pneumococci were employed indicated that the ability of a strain of penumococcus to grow in rabbit's blood is dependent on its virulence for the rabbit. The extreme susceptibility of the very young rabbit to certain strains of pneumococcus of low virulence for full-grown animals was found to be associated with an absence of pneumococcidal properties in the blood of the young rabbit. These findings suggest that the relatively susceptible animals possess the same type of defense mechanism against pneumococcus infection as do the highly pneumococcus-resistant species.

3085

Isolation and comparative action of ephedrine pseudo-ephedrine from ma-huang. (Ephedra vulgaris, var. helvetica).

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The alkaloids ephedrine and pseudo-ephedrine were isolated by Nagai[1] and Merck,[2] respectively, from *Ephedra vulgaris*. The Chinese drug Ma-huang, variously identified as *Ephedra vulgaris, Rich. var. helvetica, Hk. et Thoms.*,[3] *Ephedra equisetina, Bge.*,[4]

[1] Nagai, *Pharm. Ztg.*, 1887, xxxii, 700.

[2] Merck's Berichte, 1893, 13.

[3] Botanical Nomenclature, Commercial Press, Shanghai, 1917, 1004.

[4] Cowdry, N. H., *J. N. China Royal As. Soc.*, 1922, liii, 158.

has been recently studied by K. K. Chen and Carl F. Schmidt.[5, 6] From this they isolated the alkaloid ephedrine and conducted various physiological experiments. They gave the following physical constants for ephedrine and its salts:

Ephedrine m. p.	210° C.
Ephedrine HCl m. p.	214° C. [α] 25/D –35°.
Ephedrine H_2SO_4 m. p.	242° C.

It has been found that the basic substance isolated from Ma-huang, contains about 20 per cent of pseudo-ephedrine as well as ephedrine, the former being identical in all respects with that obtained by the action of HCl upon ephedrine. The ephedrine, having a melting point of 43°, and being considered to be laevo-rotatory up to now, is found to be dextro-rotatory in water and laevo-rotatory in alcohol. Its specific rotation suffers no change towards the action of dilute HCl, Na_2CO_3 and pepsin in acid solution. Some difference in optical activity has, however, been observed by the introduction of trypsin into its alkaline solution.

Salts of both ephedrine and pseudo-ephedrine were prepared and studied. Generally speaking the salts of ephedrine are better crystallized and less soluble in water and alcohol than those of pseudo-ephedrine. The remarkable *difference in solubility of their oxalates* in cold water affords a good means of separating ephedrine from pseudo-ephedrine, whenever a mixture of these two isomers has to be dealt with.

SALTS OF EPHEDRINE.

Hydrochloride. $C_{10}H_{15}ON.HCl$. prismatic needles, m.p. 216° C. [α] 22/D –32.5°. Easily soluble in alcohol and water. Its aqueous solution is stable at boiling temperature.

Sulphate. $C_{10}H_{15}ON$ ½ H_2SO_4. (Analysis = 7.48 per cent S) hexagonal plates, m.p. 257° [α] 22/D –30° difficulty soluble in alcohol, easily soluble in water, neutral to litmus.

Oxalate. 2 $C_{10}H_{15}ON$, $C_2H_2O_4$. prismatic needles from water m.p. 245°C. with decomposition, neutral to litmus, *only very slightly soluble in cold water.*

Phosphate. $C_{10}H_{15}ON$. H_3PO_4. (Analysis = 11.7 per cent P) crystallized from alcohol in long silky needles m.p. 178°C. acid to litmus.

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[5] Chen, K. K., and Schmidt, Carl F., *J. Pharm. Exp. Therap.*, 1924, xxiv, 339.

[6] Chen, K. K., *Am. Pharm. Assn.*, 1925, xiv, 189.

SALTS OF PSEUDO-EPHEDRINE.

Hydrochloride. $C_{10}H_{15}ON.HCl$. crystallized from alcohol in stout needles. m.p. 179-181° C. $[\alpha]$ 22/D + 58.75°, very soluble in water and in alcohol.

Sulphate. $C_{10}H_{15}ON$ ½ H_2SO_4 prismatic needles, no sharp m.p. $[\alpha]$ 22/D + 52.5°, easily soluble in water and in alcohol.

Oxalate. 2 $C_{10}H_{15}ON.C_2H_2O_4$. needles. m.p. 218° with decomposition difficulty soluble in alcohol. *Very soluble in cold* H_2O, neutral to litmus.

As far as is known pseudo-ephedrine is very similar to ephedrine in its physiological action. Previous workers have laid particular emphasis on its mydriatic effect. (See Ladenburg und Oelschlägel,[7] Lewin et Guillery,[8] and Günsberg.[9]) The latter states that, "Pseudoephedrine is a powerful mydriatic. Ten per cent solution excites the sympathetic nerve and dilates the pupil after fifteen minutes."

Reference books[10] make the general statement that like ephedrine, "pseudoephedrine is also poisonous." This statement appears to be based on scant information. A set of preliminary experiments conducted in these laboratories show that pseudoephedrine is less toxic than ephedrine for rabbits.[11] When introduced subcutaneously and intravenously the M.L.D. is about 500 milligrams and 100 milligrams respectively, which makes its toxicity relative to ephedrine about 0.645. If pseudoephedrine exhibits the same excellent clinical results obtained with ephedrine, it would be preferable for use on account of its lower toxicity.

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[7] Ladenburg und Oelschägel, *Bericht. Chem. Gesell.*, 1889, xxii, 1823.

[8] Lewin et Guillery, *Wirkungen von Arzneimitteln und Giften auf das Augen*, 1913, Berlin.

[9] Günsberg, *Virchow's Arch.*, 1891, cxxiv, 75.

[10] Henry, T. A., Plant alkaloids, 2nd edition, Philadelphia, 1924.

[11] Chen, K. K., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 404.